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(54) Title: IMPROVED ISOPRENOID PRODUCTION

(57) Abstract: Isolated polynucleotides encoding polypeptides having the activity of enzymes in the mevalonate pathway, e.g. hydroxymethylglutaryl-CoA reductase, isopetenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase, mevolante kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; are provided, useful for recombinantly producing isoprenoid compounds such as carotenoids like phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone and adonirubin. Expression vectors, cultured cells, and methods of making isoprenoid compounds are also provided.

Improved Isoprenoid Production

The present invention relates to novel polynucleotides and polypeptide sequences useful in the isoprenoid biosynthetic pathway. More particularly, the present invention provides recombinantly produced cells that exhibit improved production of zeaxanthin. Methods of making and using such cell lines are also provided.

Carotenoids are commercially important C-40 isoprenoid compounds used as nutritional supplements, pharmaceuticals and food colorants for humans and as pigments for animal feed. Currently industrially important carotenoids are produced mainly by chemical synthesis (β-carotene, canthaxanthin and astaxanthin) or extraction from natural sources (lutein from marigold, capsanthin from paprika). Production of carotenoids, however, using microorganisms has been achieved in some cases. For example, β-carotene is produced by fermentation with the fungus *Blakeslea trispora* (US 5,328,845) or by pond culture using the halotolerant alga *Dunaliella salina* [Borowitzka, J. Biotechnol. 70:313-321 (1999)]. Lycopene production has also been reported in *B. trispora* (WO 00/77234). Astaxanthin is produced by fermentation using yeast (*Phaffia rhodozyma*, (recently renamed *Xanthophyllomyces dendorous*)) (US 6,015,684) or in photobioreactors or open ponds using the alga *Haematococcus pluvialis* [Lorenz and Cysewski, Trends Biotechnol. 18:160-167 (1999); Olaizola, J. Appl. Phycol. 12:499-506 (2000)]. Such microbial production systems, however, do not produce carotenoids in amounts sufficient for economical industrial scale production.

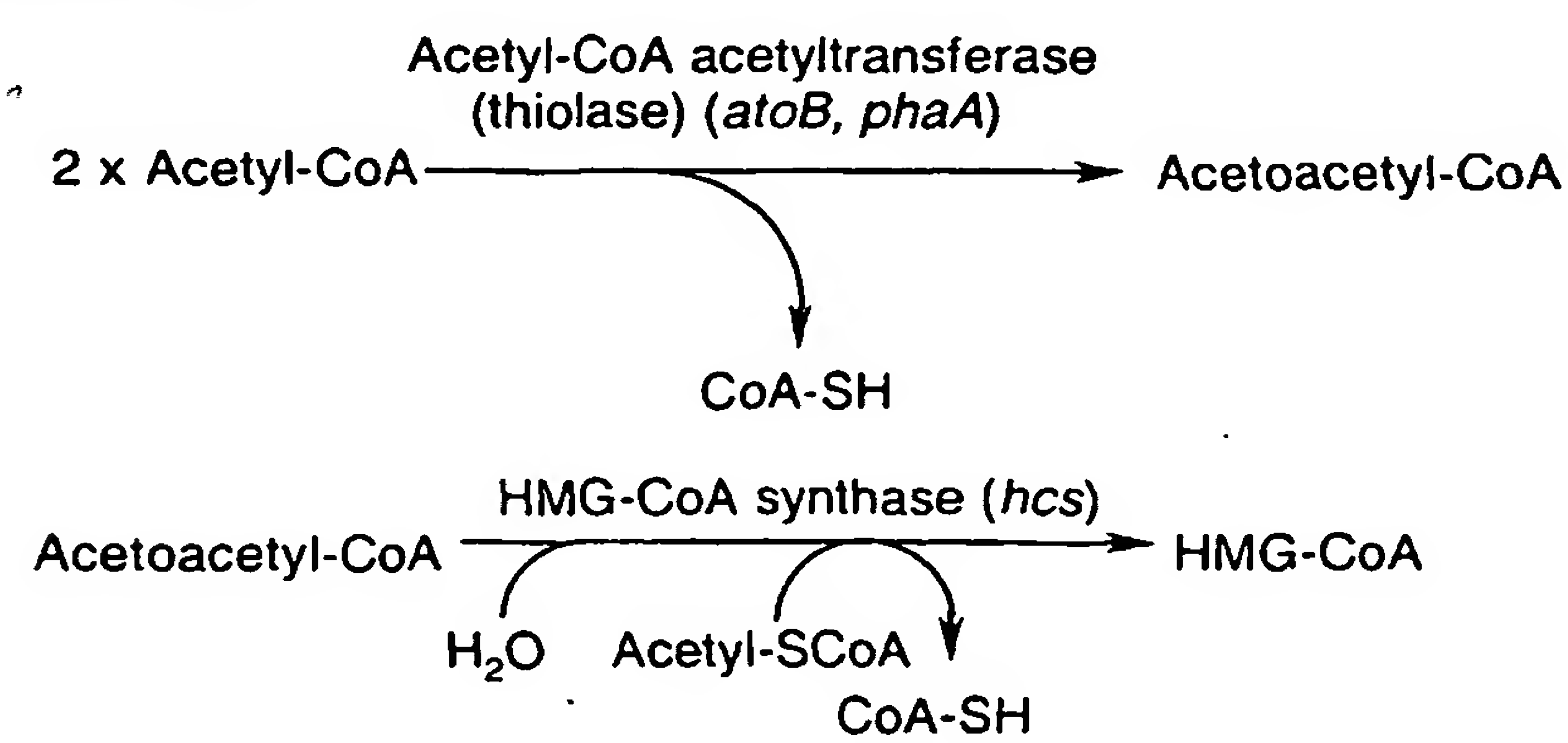
In the mid-1960's, scientists at Hoffmann-La Roche isolated several marine bacteria that produced the yellow carotenoid zeaxanthin, which has application in poultry pigmentation and in the prevention of age-related macular degeneration in humans. One bacterium, which showed promising levels of zeaxanthin production, was given the strain designation R-1512, and it was deposited at the American Type Culture Collection (ATCC, Manassas, VA, USA) as strain ATCC 21588 (US 3,891,504). Using the accepted taxonomic standards of that time (classification performed by the Eidgenössische Technische Hochschule (Zürich) and the National Collection of Industrial Bacteria, Torry Research Station (Aberdeen, Scotland)), the zeaxanthin-producing organism was classified as a member of the genus *Flavobacterium*, but no species designation was assigned.

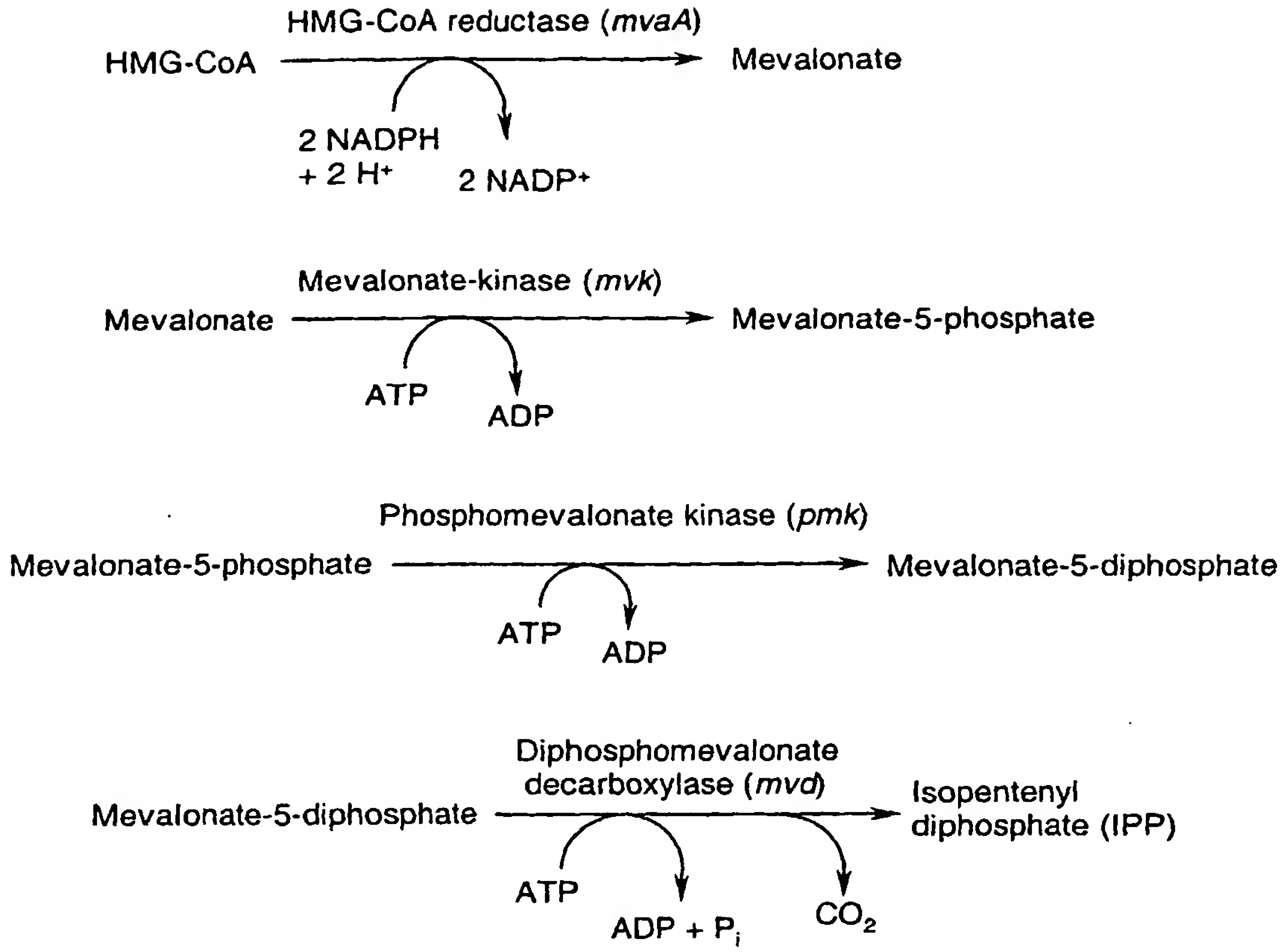
An extensive mutagenesis and screening program was subsequently conducted to isolate mutants of R-1512 with higher zeaxanthin productivities. With respect to the presently described work, two such mutants are significant. These mutants, listed in order of their zeaxanthin productivities, are R1534 and R114. A variety of other mutants have been used over the years for biochemical studies of carotenoid biosynthesis [Goodwin, Biochem. Soc. Symp. 35:233-244 (1972); McDermott et al., Biochem. J. 134:1115-1117 (1973); Britton et al., Arch. Microbiol. 113:33-37 (1977); Mohanty et al., Helvetica Chimica Acta 83:2036-2053 (2000)].

The early attempts to develop a commercially viable fermentation process for the production of zeaxanthin using classically derived mutants of strain R-1512 were not successful. However, with the advent of molecular biology, the possibility arose that higher zeaxanthin-producing strains could be developed. The first step in this direction was taken with the cloning and sequencing of the carotenoid gene cluster from strain R1534 (US 6,087,152), which is hereby incorporated by reference as if recited in full herein). US 6,087,152 discloses that the carotenoid genes were functionally expressed in *Escherichia coli* and *Bacillus subtilis* resulting in zeaxanthin production in these hosts. US 6,087,152 also disclosed that by modifying the carotenoid gene cluster or by adding a gene from an astaxanthin producing bacterium, it was possible to produce carotenoids other than zeaxanthin (EP 872,554). Moreover, EP 872,554 disclosed that carotenoid production was increased in strain R1534 by introducing cloned carotenoid gene clusters on a multi-copy plasmid.

Despite the enormous structural diversity in isoprenoid compounds, all are biosynthesized from a common C-5 precursor, isopentenyl pyrophosphate (IPP). Up until the early 1990's it was generally accepted that IPP was synthesized in all organisms via the mevalonate pathway, even though some experimental results were not consistent with this biogenic scheme [Eisenreich et al., Chemistry and Biology 5:R221-R233 (1998)].

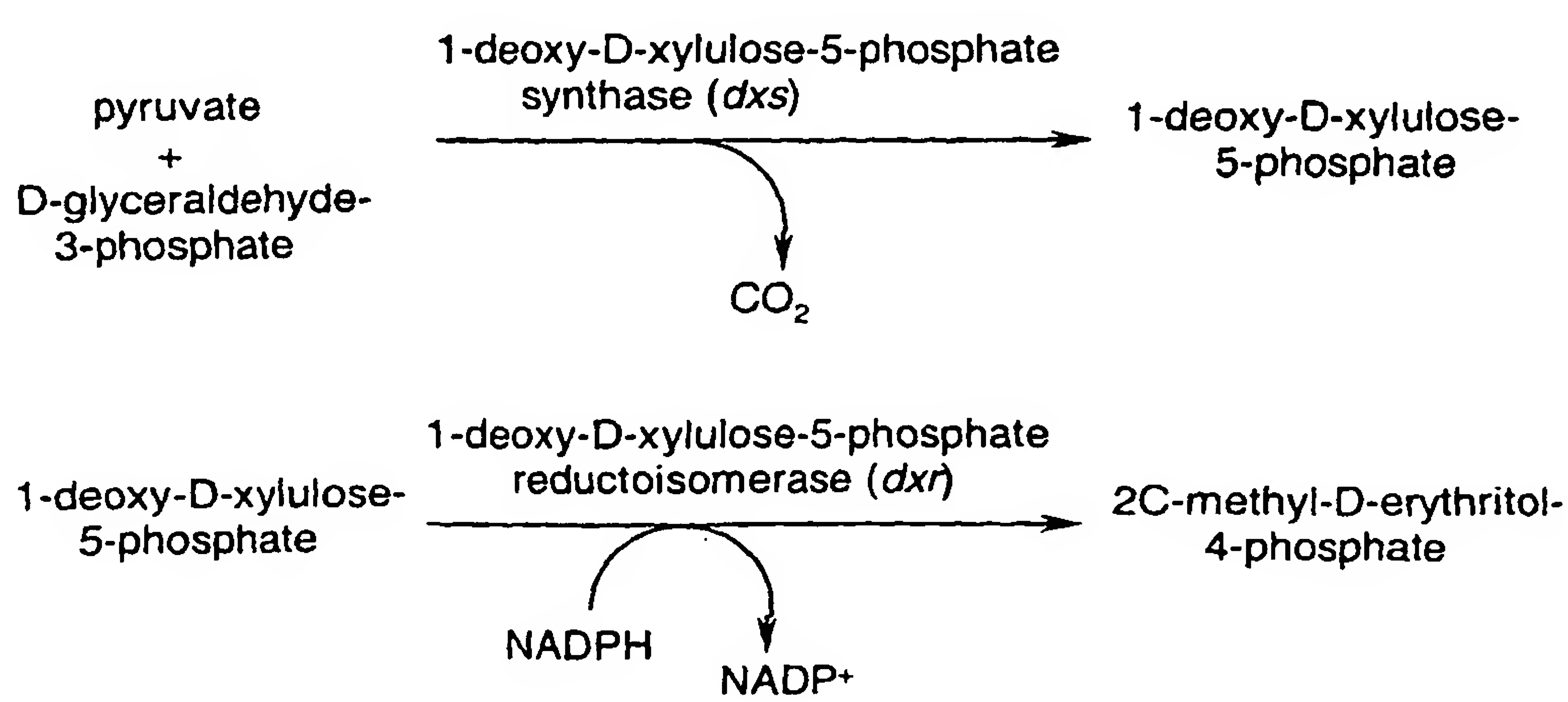
Mevalonate pathway:

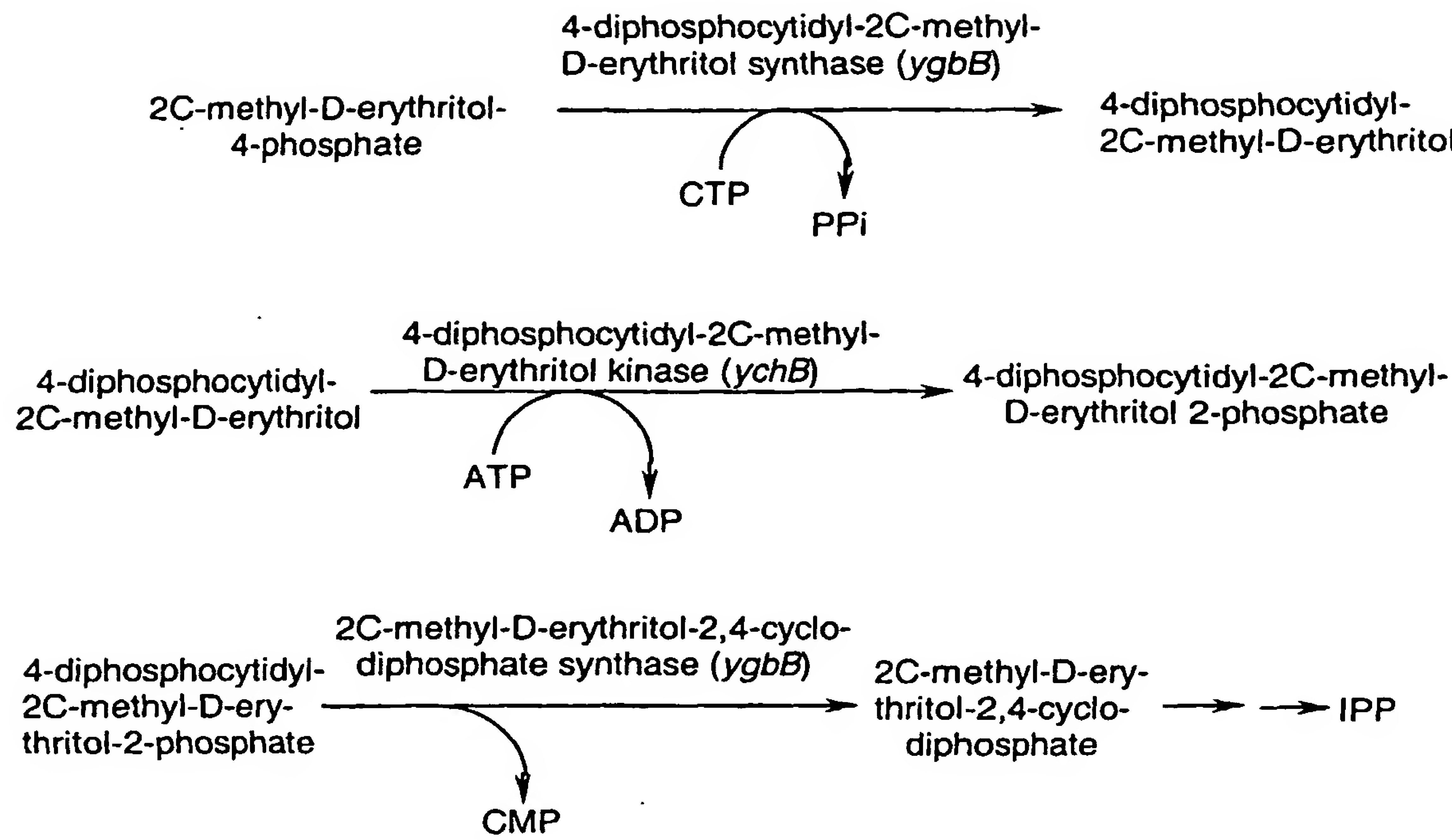




The discrepancies have since been reconciled by the discovery of an alternate pathway of IPP biosynthesis, the deoxyxylulose (DXP) pathway (Note: The alternate pathway of IPP biosynthesis has been referred to by various names in the scientific literature (DXP pathway, DOXP pathway, MEP pathway, GAP/pyruvate pathway and the non-mevalonate pathway). We use the name DXP pathway here only for the sake of simplicity). The first five reactions of the DXP pathway have been identified [Herz et al., Proc. Nat. Acad. Sci. 97:2486-2490 (2000)], but the subsequent steps leading to formation of IPP have not yet been elucidated.

DXP pathway:





McDermott et al. (supra) and Britton et al. [J. Chem. Soc. Chem. Comm. p. 27 (1979)] showed that crude extracts of zeaxanthin producing mutant strains derived from the original Roche isolates incorporated labeled mevalonate into zeaxanthin. While there was no reason to question this evidence for IPP biosynthesis via the mevalonate pathway, the work was done prior to the discovery of the DXP pathway, and it has been reported that some bacteria (*Streptomyces* species) possess both pathways for IPP synthesis and that expression of these pathways is temporally regulated [Seto et al., Tetrahedron Lett. 37:7979-7982 (1996); Dairi et al., Mol. Gen. Genet. 262:957-964 (2000)]. In addition, at present, only a small number of eubacteria have been shown to possess the mevalonate pathway for IPP synthesis. The genes encoding the enzymes of the mevalonate pathway have been cloned and sequenced from some of these bacteria [Wilding et al., J. Bacteriol. 182:4319-4327 (2000); Takagi et al., J. Bacteriol. 182:4153-4157 (2000)].

Several examples exist where the application of metabolic engineering has succeeded in altering or improving carotenoid production in microorganisms [Lagarde et al., Appl. Env. Microbiol. 66:64-72 (2000); Wang et al., Biotechnol. Bioeng. 62:235-241 (1999); Wang et al., Biotechnol. Prog. 16:922-926 (2000) (and references therein); Sandmann et al., Trends Biotechnol. 17:233-237 (2000); Misawa and Shimada, J. Biotechnol. 59:169-181 (1998); Matthews and Wurtzel, Appl. Microbiol. Biotechnol. 53:396-400 (2000); Albrecht et al., Nature Biotechnol. 18:843-846 (2000); Schmidt-Dannert et al., Nature Biotechnol. 18:750-753 (2000)]. For example, *E. coli*, a non-carotenogenic bacterium, can be engineered to produce carotenoids by introducing the cloned carotenoid (*crt*) genes from the bacteria

Agrobacterium aurantiacum, *Erwinia herbicola* or *Erwinia uredovora* (Misawa and Shimada, supra). Harker and Bramley [FEBS Lett. 448:115-119 (1999)] and Matthews and Wurtzel (supra) disclosed that carotenoid production in such engineered *E. coli* strains could be increased by over-expressing the gene coding for 1-deoxy-D-xylulose 5-phosphate synthase (DXPS), the first enzyme in the DXP pathway (*E. coli* possesses only the DXP pathway for isoprenoid biosynthesis and does not use the mevalonate pathway [Lange et al., Proc. Nat. Acad. Sci. 97:13172-13177 (2000)]. Harker and Bramley (supra) also disclosed an increase in the isoprenoid compound ubiquinone-8, in the cells overproducing DXPS. These results supported the hypothesis that limited availability of IPP, resulting from insufficient *in vivo* activity of DXPS, was limiting the production of carotenoids and other isoprenoid compounds in the engineered strains. Using a similar *E. coli* system, Kim and Keasling [Biotechnol. Bioeng. 72:408-415 (2001)] disclosed that the combined over-expression of the genes encoding DXPS and the second enzyme of the DXP pathway, DXP reductoisomerase (1-deoxy-D-xylulose-5-phosphate reductoisomerase) gave higher carotenoid production than over-expression of just the gene encoding DXPS.

All of these studies were done in *E. coli* engineered to produce carotenoids. Accordingly, one disadvantage to these studies was that the amount of carotenoids produced by these recombinant *E. coli* strains were very low compared to the amounts produced by even non-recombinant microorganisms used for industrial production of carotenoids. Furthermore, improved carotenoid production in bacteria by genetic engineering of the IPP biosynthetic pathway has only been shown in organisms that utilize the DXP pathway for IPP formation. No similar studies have been reported for bacteria that produce IPP via the mevalonate pathway.

Metabolic engineering of the mevalonate pathway to improve production of isoprenoid compounds has been reported in yeast. For example, WO 00/01649 disclosed that production of isoprenoid compounds is increased in *Saccharomyces cerevisiae* when the gene coding for 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is over-expressed. However, it has not been shown that this strategy improves isoprenoid production in bacteria, and in particular, it has not been shown that carotenoid production in bacteria can be improved by amplifying expression of mevalonate pathway genes. While it has been shown that some mevalonate pathway genes from eukaryotes [Campos et al., Biochem. J. 353:59-67 (2001)] and from the bacterium *Streptomyces* sp. strain CL190 (Takagi et al., supra) can be expressed in *E. coli*, no increase in isoprenoid production was reported in the strains.

In addition to the reactions that form IPP (via the DXP or mevalonate pathways) and the reactions that convert farnesyl pyrophosphate (FPP) to various other isoprenoids (*e.g.*, carotenoids, quinones) two other reactions are known to be involved in isoprenoid biosynthesis. IPP isomerase interconverts IPP and its isomer, dimethylallyl pyrophosphate (DMAPP). Two forms of IPP isomerase exist, the type 1 enzyme is well known in eukaryotes and some bacteria, and the newly identified type 2 enzyme that is FMN- and NADP(H)-dependent [Kaneda et al., Proc. Nat. Acad. Sci. 98:932-937 (2001)].

Several reports disclose that in *E. coli* engineered to produce carotenoids, amplification of native or heterologous type 1 IPP isomerase (*idi*) genes stimulates carotenoid production [Kajiwara et al., Biochem. J. 324:421-426 (1997); Verdoes and van Ooyen, Acta Bot. Gallica 146:43-53 (1999); Wang et al., supra]. In one report (Wang et al, supra), it was further disclosed that over-expression of the *ispA* gene, encoding FPP synthase (Farnesyl diphosphate synthase) increased carotenoid production in an engineered carotenogenic strain of *E. coli* when combined with over-expression of the *idi* and *crtE* (GGPP synthase/Geranylgeranyl diphosphate synthase) genes. As is the case for the pathway of IPP biosynthesis, however, it has not been shown that over-expression of genes coding for IPP isomerase or FPP synthase improves carotenoid production in a naturally carotenogenic microorganism. Also, the levels of carotenoids produced in the *E. coli* strains described above are very low, and it has not been shown that these strategies work in an industrial microorganism where carotenoid production was already high.

In sum, there is no prior evidence that increased expression of gene(s) coding for enzymes of the mevalonate pathway can improve production of carotenoids in naturally carotenogenic bacteria or in naturally non-carotenogenic bacteria engineered to be carotenogenic.

One embodiment of the present invention is an isolated polypeptide that includes an amino acid sequence selected from the following group: (a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43; (b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45; (c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47; (d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49; (e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51; (f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53; (g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues; (h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of HMG-CoA reductase; isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA

synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; (i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, isopentenyl diphosphate isomerase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and (j) a conservatively modified variant of SEQ ID NOs:43, 45, 47, 49, 51 or 53.

As noted above, the present invention includes SEQ ID Nos: 43, 45, 47, 49, 51, and 53, which are polypeptide sequences that correspond to the following enzymes of the mevalonate pathway: hydroxymethyl glutaryl CoA (HMG-CoA) reductase, isopentenyl diphosphate (IPP) isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, respectively. The present invention also includes at least 30 contiguous amino acids of each identified sequence or a sufficient number of contiguous amino acids to define a biologically active molecule.

The present invention also includes fragments of a polypeptide selected from SEQ ID NOs: 43, 45, 47, 49, 51, and 53. The fragment should be at least about 30 amino acids in length but must have the activity of the identified polypeptide, *e.g.*, in the case of SEQ ID NO:43, a fragment thereof that falls within the scope of the present invention has the activity of HMG-CoA reductase. As used herein, a measure of activity of the respective fragments is set forth in Example 1. A fragment having an activity above background in the assays set forth in Example 1 is considered to be biologically active and within the scope of the present invention.

The present invention also includes an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions, as defined above, to a hybridization probe that contains at least 30 contiguous nucleotides of SEQ ID NO:42 (*i.e.*, the mevalonate operon) or a complement of SEQ ID NO:42. The polynucleotide must encode at least one of the enzymes in the mevalonate pathway. For purposes of the present invention, a "hybridization probe" is a polynucleotide sequence containing from about 10-9066 nucleotides of SEQ ID NO:42.

In this embodiment, the isolated polypeptide may have the amino acid sequence of SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51 or SEQ ID NO:53. Alternatively, the isolated polypeptide may contain about 30 contiguous amino acids selected from an area of the respective amino acids sequences that have the least

identity when compared to an enzyme with the same function from different species. Thus, for example, a polypeptide of the present invention may include amino acids 68-97 of SEQ ID NO:43, 1-30 of SEQ ID NO:45, 269-298 of SEQ ID NO:47, 109-138 of SEQ ID NO:49, 198-227 of SEQ ID NO:51 or 81-110 of SEQ ID NO:53.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159; (b) at least 30 contiguous amino acid residues of SEQ ID NO:159; (c) an amino acid sequence of a fragment of SEQ ID NO:159, the fragment having the activity of farnesyl-diphosphate synthase (FPP synthase); (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *ispA* gene (*i.e.*, nucleotides 295-1158 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and (e) conservatively modified variants of SEQ ID NO:159.

Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes FPP synthase, *i.e*, residues 1-287 of SEQ ID NO:159, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:159 that has FPP synthase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *ispA* gene (*i.e.*, nucleotides 295-1158 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has FPP synthase activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:159.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from the following group: (a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160; (b) at least 30 contiguous amino acid residues of SEQ ID NO:160; (c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS); (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of DXPS; and (e) conservatively modified variants of SEQ ID NO:160.

Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes DXPS, *i.e*, residues 1-142 of SEQ ID NO:160, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:160 that has DXPS activity as measured by as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the DXPS gene (*i.e.*, nucleotides 1185-1610 of SEQ ID NO:157) or a complement thereof, wherein the polypeptide has DXPS activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:160.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178; (b) at least 30 contiguous amino acid residues of SEQ ID NO:178; (c) an amino acid sequence of a fragment of SEQ ID NO:178, the fragment having the activity of acetyl-CoA acetyltransferase; (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *phaA* gene (*i.e.*, nucleotides 1-1179 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has the activity of acetyl-CoA acetyltransferase, and (e) conservatively modified variants of SEQ ID NO:178.

Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes acetyl-CoA acetyltransferase, *i.e*, residues 1-143 of SEQ ID NO:178, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:178 that has acetyl-CoA acetyltransferase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *phaA* gene (*i.e.*, nucleotides 1-1170 of SEQ ID NO:177), or a complement thereof, wherein the polypeptide has the acetyl-CoA acetyltransferase activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:178.

Another embodiment of the invention is an isolated polypeptide having an amino acid sequence selected from: (a) an amino acid sequence shown as residues 1 to 240 of SEQ ID

NO:179; (b) at least 30 contiguous amino acid residues of SEQ ID NO:179; (c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO:179, the fragment having the activity of acetoacetyl-CoA reductase; (d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe containing at least 30 consecutive nucleotides of the *phaB* gene (*i.e.*, nucleotides 1258-1980 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has the activity of acetoacetyl-CoA reductase; and (e) conservatively modified variants of SEQ ID NO:179.

Thus, in this embodiment the amino acid may be encoded by the entire open reading frame that encodes acetoacetyl-CoA reductase, *i.e*, residues 1-240 of SEQ ID NO:179, at least 30 contiguous residues thereof, or a fragment of SEQ ID NO:179 that has acetoacetyl-CoA reductase activity as measured by the assay set forth in Example 1. Furthermore, this embodiment of the invention also includes amino acid sequence(s) encoded by polynucleotide(s) that hybridize under stringent conditions, as defined above, to a hybridization probe that includes at least 30 consecutive nucleotides of the *phaB* gene (*i.e.*, nucleotides 1258-1980 of SEQ ID NO:177) or a complement thereof, wherein the polypeptide has acetoacetyl-CoA reductase activity as defined above.

In a preferred embodiment, the polypeptide has the amino acid sequence of SEQ ID NO:179.

The terms "polypeptide," "polypeptide sequence," "amino acid," and "amino acid sequence" are used interchangeably herein, and mean an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, as well as naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of any of the polypeptides defined herein which are at least about 30 amino acids in length and which retain some biological activity or immunological activity of the polypeptide in question. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

With respect to polypeptides, the term "isolated" means a protein or a polypeptide that has been separated from components that accompany it in its natural state. A monomeric protein is isolated when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. An isolated protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein

purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, using HPLC or other means well known in the art may provide higher resolution for purification.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic polypeptide, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of the mevalonate operon (SEQ ID NO:42), variants of SEQ ID NO:42 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of SEQ ID NO:42. The variants and fragments of SEQ ID NO:42 must encode a polypeptide having an activity selected from: HMG-CoA reductase, isopentenyl diphosphate isomerase activity, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe, the nucleotide sequence of which consists of from about 10 to about 9066 nucleotides of SEQ ID NO:42, preferably at least 30 contiguous nucleotides of SEQ ID NO:42, or a complement of such sequences, which polynucleotide encodes a polypeptide having an activity selected from: HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase.

This embodiment also includes isolated polynucleotide sequences spanning the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878. Fragments of these sequences are also within the scope of the invention, so long as they encode a polypeptide having HMG-CoA reductase activity, isopentenyl diphosphate isomerase activity, HMG-CoA synthase activity, mevalonate kinase activity, phosphomevalonate kinase activity, and diphosphomevalonate decarboxylase activity, respectively.

This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe selected from a nucleotide sequence which consists of at least 30 contiguous nucleotides of the following residues of SEQ ID

NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878 or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA reductase activity, isopentenyl diphosphate isomerase activity, HMG-CoA synthase activity, mevalonate kinase activity, phosphomevalonate kinase activity, or diphospho-mevalonate decarboxylase activity, respectively.

Preferably, the isolated polynucleotide consists of nucleotides 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887 or 7880 to 8878 of SEQ ID NO:42.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of SEQ ID NO:157 that encode a polypeptide having FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or the activity of XseB. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of SEQ ID NO:157, wherein the polynucleotide encodes a polypeptide having FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or the activity of XseB.

Preferably, the isolated polynucleotide consists of nucleotides 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157.

An isolated polynucleotide sequence is also provided that has a nucleotide sequence selected from the following group: nucleotides spanning positions 59-292 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 59-292 of SEQ ID NO:157 that encode a polypeptide having a function of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 59-292 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having a function of XseB.

Preferably, the isolated polynucleotide consists of nucleotides 59 to 292 of SEQ ID NO:157.

An isolated polynucleotide sequence is also provided that has a nucleotide sequence selected from the following group: nucleotides spanning positions 295-1158 of SEQ ID

NO:157, variants of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 that encode a FPP synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 295-1158 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having FPP synthase activity.

Preferably, the isolated nucleotide sequence consists of nucleotides 295-1158 of SEQ ID NO:157.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 that encode a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 1185-1610 of SEQ ID NO:157, or a complement thereof, wherein the polynucleotide encodes a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity.

Preferably, the isolated polynucleotide consists of nucleotides 1185 to 1610 of SEQ ID NO:157.

Another embodiment of the invention is an isolated polynucleotide sequence having the nucleotide sequence of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions, as defined above, to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:177, or a complement thereof, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase.

In this embodiment the isolated polynucleotide sequence may include nucleotides 1 to 1170 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of SEQ ID NO:177 that encode a polypeptide having acetyl-CoA acetyltransferase activity. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of nucleotides 1 to 1170 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetyl-CoA acetyl-transferase activity.

Preferably, the isolated polynucleotide sequence consists of nucleotides 1-1170 of SEQ ID NO:177.

In this embodiment, the isolated polynucleotide sequence may alternatively be nucleotides 1258-1980 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more sub-stitutions according to the *Paracoccus* sp. strain 1534 codon usage table (*see* Table 14) or fragments of SEQ ID NO:177 that encode a polypeptide having acetoacetyl-CoA reductase activity. This embodiment also includes polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of nucleotides 1258-1980 of SEQ ID NO:177, or a com-plement thereof, wherein the polynucleotide encodes a polypeptide having acetoacetyl-CoA reductase activity.

Preferably, the isolated polynucleotide consists of nucleotides 1258-1980 of SEQ ID NO:177.

In another embodiment of the invention, the isolated polynucleotide sequence has a nucleotide sequence selected from SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and combinations thereof. As used herein, the phrase "and combinations thereof" when used in reference to nucleotide sequences means that any combination of the recited sequences may be combined to form the isolated polynucleotide sequence. Moreover, in the present invention, multiple copies of the same sequence, *i.e.*, concatamers may be used. Likewise, and as set forth in more detail below, multiple copies of plasmids containing the same polynucleotide sequence may be transferred into suitable host cells.

As used herein, an "isolated" polynucleotide (*e.g.*, an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native sequence or polypeptide, *e.g.*, ribosomes, polymerases, many other

genome sequences and proteins. The term embraces a polynucleotide that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

The phrase “nucleic acid sequence” refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role.

An “expression control sequence” is defined as an array of nucleic acid control sequences that direct transcription of an operably linked nucleic acid. An example of such an expression control sequence is a “promoter.” Promoters include necessary nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A “constitutive” promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term “operably linked” refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

A polynucleotide sequence is “heterologous to” an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, a promoter operably linked to a heterologous coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from any naturally occurring allelic variants.

In the case of both expression of transgenes and inhibition of endogenous genes (*e.g.*, by antisense, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only "substantially identical" to a sequence of the gene from which it was derived.

In the case where the inserted polynucleotide sequence is transcribed and translated to produce a functional polypeptide, one of skill will recognize that because of codon degeneracy a number of polynucleotide sequences will encode the same polypeptide. These vari-

ants are specifically within the scope of the present invention. In addition, the present invention specifically includes those sequences that are substantially identical (determined as described below) to each other and that encode polypeptides that are either mutants of wild type polypeptides or retain the function of the polypeptide (*e.g.*, resulting from conservative substitutions of amino acids in the polypeptide). In addition, variants can be those that encode dominant negative mutants as described below.

Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. When percentage of sequence identity is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (*e.g.*, charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated according to, *e.g.*, the algorithm of Meyers & Miller, Computer Applic. Biol. Sci. 4:11-17 (1988), *e.g.*, as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif., USA).

The phrase "substantially identical," in the context of two nucleic acids or polypeptides, refers to sequences or subsequences that have at least 60%, preferably 80%, most preferably 90-95%, nucleotide or amino acid residue identity when aligned for maximum correspondence over a comparison window as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. This

definition also refers to a sequence of which the complement of that sequence hybridizes to the test sequence.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Nat'l, Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection.

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle, J. Mol. Evol. 35:351-360 (1987). The method used is similar to the method described by Higgins and Sharp, CABIOS 5:151-153 (1989). The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and

their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm [Altschul et al., J. Mol. Biol. 215:403-410 (1990)]. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences [see, *e.g.*, Karlin and Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)]. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences,

or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acid codons encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, or substitutions to a peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids (*i.e.* less than 20%, such as 15%, 10%, 5%, 4%, 3%, 2% or 1%) in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

Alanine (A), Serine (S), Threonine (T);
Aspartic acid (D), Glutamic acid (E);
Asparagine (N), Glutamine (Q);
Arginine (R), Lysine (K);
Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
Phenylalanine (F), Tyrosine (Y), Tryptophan (W). (see, *e.g.*, Creighton, Proteins (1984)).

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two

molecules or their complements hybridize to each other under stringent conditions, as described below.

The phrase "specifically hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (*e.g.*, total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target sequence, typically in a complex mixture of nucleic acid sequences, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of Principles of Hybridization and the Strategy of Nucleic Acid Assays" (1993). Generally, highly stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Low stringency conditions are generally selected to be about 15-30°C below the T_m. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0M sodium ion, typically about 0.01 to 1.0M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions.

In the present invention, genomic DNA or cDNA containing nucleic acids of the invention can be identified in standard Southern blots under stringent conditions using the nucleic

acid sequences disclosed here. For the purposes of this disclosure, suitable stringent conditions for such hybridizations are those which include hybridization in a buffer of 40% formamide, 1M NaCl, 1% sodium dodecyl sulfate (SDS) at 37°C, and at least one wash in 0.2X SSC at a temperature of at least about 50°C, usually about 55°C to about 60°C, for 20 minutes, or equivalent conditions. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

A further indication that two polynucleotides are substantially identical is if the reference sequence, amplified by a pair of oligonucleotide primers, can then be used as a probe under stringent hybridization conditions to isolate the test sequence from a cDNA or genomic library, or to identify the test sequence in, *e.g.*, a northern or Southern blot.

The present invention also includes expression vectors as defined above. The expression vectors include one or more copies of each of the polynucleotide sequences set forth above. The expression vectors of the present invention may contain any of the polynucleotide sequences defined herein, such as for example SEQ ID NO:42, or the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177. The expression vectors may contain combinations of the polynucleotide sequences identified herein, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

The polynucleotide sequences in the expression vectors may optionally be operably linked to an expression control sequence as defined above and exemplified in the Examples.

The present invention also includes for example, the following expression vectors: pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His–*mvaA*, pDS-His–*idi*, pDS-His-*hcs*, pDS-His–*mvk*, pDS-His–*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-P*crtE*-*crtE*-3, pBBR-tK-P*crtE*-*mva*A, pBBR-tK-P*crtE*-*idi*, pBBR-tK-P*crtE*-*hcs*, pBBR-tK-P*crtE*-*mvk*, pBBR-tK-P*crtE*-*pmk*, pBBR-tK-P*crtE*-*mvd*, pBBR-K-P*crtE*-*mvaA*-*crtE*-3, pDS-His-*phaA*, pBBR-K-P*crtE*-*crtW*, pBBR-K-P*crtE*-*crtWZ*, pBBR-K-P*crtE*-*crtZW*, and combinations thereof. These expression vectors are defined in more detail in the examples below. Moreover, the present invention also includes any expression vector that contains one of the sequences defined herein, which expression vector is used to express an isoprenoid compound, such as a carotenoid, preferably zeaxanthin, in a suitable host cell.

As used herein, the phrase "expression vector" is a replicatable vehicle that carries, and is capable of mediating the expression of, a DNA sequence encoding the polynucleotide sequences set forth herein.

In the present context, the term "replicatable" means that the vector is able to replicate in a given type of host cell into which it has been introduced. Immediately upstream of the polynucleotide sequence(s) of interest, there may be provided a sequence coding for a signal peptide, the presence of which ensures secretion of the encoded polypeptide expressed by host cells harboring the vector. The signal sequence may be the one naturally associated with the selected polynucleotide sequence or of another origin.

The vector may be any vector that may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication; examples of such a vector are a plasmid, phage, cosmid or mini-chromosome. Alternatively, the vector may be one which, when introduced in a host cell, is integrated in the host cell genome and is replicated together with the chromosome(s) into which it has been integrated. Examples of suitable vectors are shown in the examples. The expression vector of the invention may carry any of the DNA sequences of the invention as defined below and be used for the expression of any of the polypeptides of the invention defined below.

The present invention also includes cultured cells containing one or more of the polynucleotide sequences and/or one or more of the expression vectors disclosed herein. As used herein, a "cultured cell" includes any cell capable of growing under defined conditions and expressing one or more of polypeptides encoded by a polynucleotide of the present invention. Preferably, the cultured cell is a yeast, fungus, bacterium, or alga. More preferably, the cultured cell is a *Paracoccus, Flavobacterium, Agrobacterium, Alcaligenes, Erwinia, E. coli* or *B. subtilis*. Even more preferably, the cell is a *Paracoccus*, such as for example, R-1506, R-1512, R1534 or R114. The present invention also includes the progeny of any of the cells identified herein that express a polypeptide disclosed herein. In the present invention, a cell is a progeny of another cell if its AFLP DNA fingerprint is indistinguishable using the conditions set forth in Example 2 from the fingerprint of the putative parental cell.

Thus, the cultured cells according to the present invention may contain, for example, SEQ ID NO:42, or the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687

to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177. These sequences may be transferred to the cells alone or as part of an expression vector. These sequences also may optionally be operatively linked to expression control sequence(s). The cultured cells may also contain combinations of the polynucleotide sequences identified herein, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

The cultured cells according to the present invention may further contain polynucleotides that encode one or more enzymes in the carotenoid biosynthetic pathway. For example, the cultured cells according to the present invention may contain one or more copies of SEQ ID NOs:180, 182, and 184 alone or in combination with any of the polynucleotide sequences identified herein. Thus, the polynucleotide sequences disclosed herein may be transferred into a cultured cell alone or in combination with another polynucleotide sequence that would provide enhanced production of the target isoprenoid compound, such as, for example, carotenoids like zeaxanthin or astaxanthin. In this regard, the present invention includes the use of any polynucleotide encoding, for example, a polypeptide involved in carotenoid biosynthesis, such as GGPP synthase, β-carotene-β4-oxygenase (ketolase), and/or β-carotene hydroxylase. In addition, combinations of polynucleotides encoding polypeptides involved in carotenoid biosynthesis may be used in combination with one or more of the polynucleotides identified herein on the same or different expression vectors. Such constructs may be transferred to a cultured cell according to the present invention to provide a cell that expresses an isoprenoid of interest.

For example, a cultured cell according to the present invention may contain one or more of the following expression vectors: pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His–*mvaA*, pDS-His–*idi*, pDS-His-*hcs*, pDS-His–*mvk*, pDS-His–*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-P*crtE*-*crtE*-3, pBBR-tK-P*crtE*-*mva*A, pBBR-tK-P*crtE*-*idi*, pBBR-tK-P*crtE*-*hcs*, pBBR-tK-P*crtE*-*mvk*, pBBR-tK-P*crtE*-*pmk*, pBBR-tK-P*crtE*-*mvd*, pBBR-K-P*crtE*-*mvaA*-*crtE*-3, pDS-His-*phaA*, pBBR-K-P*crtE*-*crtW*, pBBR-K-P*crtE*-*crtWZ*, pBBR-K-P*crtE*-*crtZW*, and combinations thereof.

Another embodiment of the invention is a method of producing a carotenoid. In this method, a cultured cell as defined above is cultured under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence as defined above. Culture conditions that permit expression of a polypeptide are provided in the Examples

below, but may be modified, if required, to suit the particular intended use. The carotenoid is then isolated from the cell or, if secreted, from the medium of the cell.

In the present invention, a "carotenoid" includes the following compounds: phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof. Preferably, the carotenoid is zeaxanthin.

Another embodiment of the invention is a method of making a carotenoid-producing cell. This method includes (a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and (b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.

As used herein, the phrase "an enzyme in the mevalonate pathway" means the enzymes involved in the mevalonate pathway for IPP biosynthesis and encoded by the *atoB* or *phaA*, *hcs*, *mvaA*, *mvk*, *pmk*, and *mvd* genes. For purposes of the present invention, an enzyme is "expressed in the cell" if it is detected using any one of the activity assays set forth in Example 1. Assays for detecting the production of a carotenoid are well known in the art. Examples 1, 11, and 12 provide typical assay procedures for identifying the presence of zeaxanthin, lycopene, and astaxanthin, respectively. In a similar manner, assays for the other carotenoids may be used to detect the presence in the cell or medium of *e.g.* phytoene, canthaxanthin, adonixanthin, cryptoxanthin, echinenone, and adonirubin.

Thus, this method may be used to make the following exemplary carotenoids: phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof. In this method, zeaxanthin is the preferred carotenoid.

This method includes producing cells capable of producing a carotenoid at a level that is about 1.1-1,000 times, preferably about 1.5-500 times, such as about 100 times or at least 10 times, the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.

In this method, the cell produces from about 1 mg/L to about 10 g/L of a carotenoid. It is preferred that the cell produces from about 100 mg/L to about 9 g/L, such as, for example, from about 500mg/L to about 8 g/L, or from about 1 g/L to about 5 g/L, of a carotenoid.

In this method, the cell may be selected from a yeast, fungus, bacterium, and alga. Preferably, the cell is a bacterium selected from *Paracoccus, Flavobacterium, Agrobacterium, Alcaligenes, Erwinia, E. coli*, and *B. subtilis*. More preferably, the bacterium is a *Paracoccus*.

In this method, the cell may be a mutant cell. As used herein, a "mutant cell" is any cell that contains a non-native polynucleotide sequence or a polynucleotide sequence that has been altered from its native form (*e.g.*, by rearrangement or deletion or substitution of from 1-100, preferably 20-50, more preferably less than 10 nucleotides). Such a non-native sequence may be obtained by random mutagenesis, chemical mutagenesis, UV-irradiation, and the like. Preferably, the mutation results in the increased expression of one or more genes in the mevalonate pathway that results in an increase in the production of a carotenoid, such as zeaxanthin. Methods for generating, screening for, and identifying such mutant cells are well known in the art and are exemplified in the Examples below. Examples of such mutants are R114 or R1534. Preferably, the mutant cell is R114.

≙ mev-operon

In this method, the polynucleotide sequence is SEQ ID NO:42, or the following residues of SEQ ID NO:42: 2622 to 3644, 3641 to 4690, 4687 to 5853, 5834 to 6970, 6970 to 7887, 7880 to 8878, as well as residues 59-292, 295-1158 or 1185-1610 of SEQ ID NO:157 and residues 1-1170 or 1258-1980 of SEQ ID NO:177. These sequences may be used in this method alone or as part of an expression vector. These sequences also may optionally be operatively linked to expression control sequence(s). In this method, combinations of the polynucleotide sequences identified herein may be used, such as for example, SEQ ID NO:42, SEQ ID NO:157, and SEQ ID NO:177.

Examples of expression vector that may be selected for use in this method include pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His–*mvaA*, pDS-His–*idi*, pDS-His-*hcs*, pDS-His–*mvk*, pDS-His–*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-P*crtE*-*crtE*-3, pBBR-tK-P*crtE-mvaA*, pBBR-tK-P*crtE-idi*, pBBR-tK-P*crtE-hcs*, pBBR-tK-P*crtE*-*mvk*, pBBR-tK-P*crtE-pmk*, pBBR-tK-P*crtE-mvd*, pBBR-K-P*crtE-mvaA-crtE*-3, pDS-His-*phaA*, pBBR-K-P*crtE-crtW*, pBBR-K-P*crtE-crtWZ*, pBBR-K-P*crtE-crtZW*, and combinations thereof.

In this method, the polynucleotide sequence is introduced into the cell using any conventional means. Examples of suitable methods for introducing a polynucleotide sequence into a cell include transformation, transduction, transfection, lipofection, electroporation [*see e.g.*, Shigekawa and Dower, Biotechniques 6:742-751 (1988)], conjugation [*see e.g.*, Koehler and Thorne, Journal of Bacteriology 169:5771-5278 (1987)], and biolistics.

The use of conjugation to transfer a polynucleotide sequence, such as in the form of an expression vector, into recipient bacteria is generally effective, and is a well-known procedure. (e.g. US 5,985,623). Depending on the strain of bacteria, it may be more common to use transformation of competent cells with purified DNA.

Known electroporation techniques (both *in vitro* and *in vivo*) function by applying a brief high voltage pulse to electrodes positioned around the treatment region. (e.g. US 6,208,893). The electric field generated between the electrodes causes the cell membranes to temporarily become porous, whereupon molecules of the implant agent enter the cells. In known electroporation applications, this electric field comprises a single square wave pulse on the order of 1000 V/cm of about 100 μs duration. Such a pulse may be generated, for example, in known applications of the Electro Square Porator T820, made by the BTX Division of Genetronics, Inc.

Biolistics is a system for delivering polynucleotides into a target cell using microprojectile bombardment techniques. An illustrative embodiment of a method for delivering polynucleotides into target cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with cultured target cells. The screen disperses the particles so that they are not delivered to the target cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be bombarded reduces the size of projectiles aggregate and may contribute to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.

For the bombardment, cells in suspension are preferably concentrated on filters or solid culture medium. Alternatively, other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the microprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of these well-known techniques one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a focus which express the exogenous gene product 48 hours post-bombardment often range from 1 to 10 and average 1 to 3.

In bombardment transformation, one may optimize the prebombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment are important in this technology. Physical factors are those that involve manipulating the polynucleotide/-

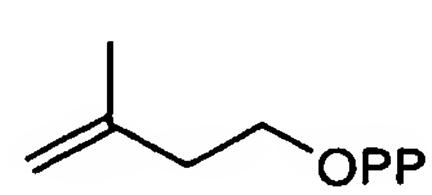
microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment, and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids.

Accordingly, it is contemplated that one may wish to adjust various of the bombardment parameters in small-scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance, and helium pressure. One may also minimize the trauma reduction factors (TRFs) by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

The methods of particle-mediated transformation is well known to those of skill in the art. E.g. US 5,015,580 (specifically incorporated herein by reference) describes the transformation of soybeans using such a technique.

Another embodiment of the invention is a method for engineering a bacterium to produce an isoprenoid compound. Such a bacterium is made by (a) culturing a parent bacterium in a medium under conditions permitting expression of an isoprenoid, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid than the parent bacteria; (b) introducing into the mutant bacterium an expression vector containing a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and (c) selecting a bacterium that contains the expression vector and produces at least about 1.1 times more of an isoprenoid than the mutant in step (a).

In this embodiment, an isoprenoid compound means a compound structurally based on isopentenyl diphosphate (IPP) units of the formula:



Such compounds include the hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes (*e.g.*, phytosterols, phytoestrogens, phytoecdysones, estrogens, phytoestrogens),

tetraterpenes (carotenoids), and polyterpenes. Preferably, the isoprenoid is a carotenoid, such as for example, one of the carotenoids identified above, in particular zeaxanthin.

The bacterium may be any bacterium that is capable of producing an isoprenoid compound using the processes disclosed herein. Preferably, the bacterium is a *Paracoccus, Flavobacterium, Agrobacterium, Alcaligenes, Erwinia, E. coli*, or *B. subtilis*. Even more preferably, the bacterium is a *Paracoccus*. Preferably, the parent bacterium is R-1506 or R 1512, and the mutant bacterium is R1534 or R114, preferably R114.

The bacterium is cultured in a media and under conditions that are optimized for the production of the isoprenoid. The selection of media and culture conditions are well within the skill of the art. The assays set forth in Examples 1, 11, and 12 provide exemplary methods for measuring the presence of certain carotenoids in a culture media. By optimizing the culture conditions and measuring for the production of the target isoprenoid, the culturing and selection of a mutant that meets the specific production parameters recited herein may be met. In this way, a mutant bacterium producing from about 1.1-1,000 times more of an isoprenoid than the parent bacterium may be selected. Preferably, the mutant bacterium produces from about 1.5-500 times more of an isoprenoid than the parent bacterium, such as for example, at least about 100 times or at least about 10 times more of an isoprenoid than the parent bacterium. That bacterium is then cultured and used in subsequent steps.

After selecting the mutant bacterium that produces the desired level of an isoprenoid, an expression vector is introduced into the bacterium using any of the methods set forth above or described in the examples. Any of the expression vectors defined herein may be introduced into the mutant cell. Preferably, the expression vector contains SEQ ID NO:42.

Once the expression vector is introduced into the mutant bacteria, a stable transformant is selected that produces at least about 1.1 times, such as about 5 to about 20 times, more of an isoprenoid than the untransformed mutant. The selected transformant is then cultured under conditions suitable for isoprenoid production, and then the isoprenoid is isolated from the cell or the culture medium.

A further step in this method is introducing a mutation into the mutant bacterium that results in the increased production of an isoprenoid compound by the bacterium. The mutation may be selected from at least one of the following: inactivating the polyhydroxyalkanoate (PHA) pathway, increasing expression of acetyl-CoA acetyltransferase, increasing expression of FPP synthase, increasing expression of an enzyme in a carotenoid

biosynthetic pathway, and increasing the expression of an enzyme for converting isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP).

The inactivating of the PHA pathway may be achieved by selecting for a mutant bacterium that does not express a polypeptide encoded by *phaB* (nucleotide positions 1258-1980 of SEQ ID NO:177) or by disrupting expression of the wild type *pha*B gene by homologous recombination using SEQ ID NO:177 or fragments thereof.

In this method, increasing expression of acetyl-CoA acetyltransferase may be achieved by introducing into the mutant bacterium a vector containing a polynucleotide sequence represented by SEQ ID NO:175 or nucleotide positions 1-1170 of SEQ ID NO:177 operably linked to an expression control sequence. In this method, increasing expression of FPP synthase may be achieved by introducing into the mutant bacterium a vector containing a polynucleotide sequence represented by nucleotides 295-1158 of SEQ ID NO:157 operably linked to an expression control sequence. In this method, increasing expression of a carotenoid gene may be achieved by introducing into the mutant bacterium a vector comprising a polynucleotide sequence that encodes one or more enzymes in the carotenoid biosynthetic pathway, such as for example a polynucleotide sequence selected from the group consisting of SEQ ID NOs:180, 182, and 184 operably linked to an expression control sequence.

In this method, it is preferred that the isoprenoid compound is isopentenyl diphosphate (IPP). It is also preferred that the isoprenoid compound is a carotenoid, such as for example, phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) a sequence similiarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%; (b) a homology to R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C; (c) a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and (d) an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534, R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (*i.e.*, a-d). However, any combination of the characteristics a-d, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes; (b) an inability to use adonitol, i-erythritol, gentiobiose, β-methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and (c) an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are also fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (*i.e.*, a-c). However, any combination of the characteristics a-c, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

Another embodiment of the invention is a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics: (a) an ability to grow at 40°C; (b) an ability to grow in a medium having 8% NaCl; (c) an ability to grow in a medium having a pH of 9.1; and (d) a yellow-orange colony pigmentation, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

Methods for determining each of these characteristics are also fully set forth in Example 2, and it is contemplated when these methods are used that microorganisms meeting the above criteria will be readily identifiable. It is preferred that a microorganism of the present invention have each characteristic set forth above (*i.e.*, a-d). However, any combination of the characteristics a-d, which provides sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534,

and R-1506, with the exception of *Paracoccus* sp. (MBIC3966) is also within the scope of the invention.

A microorganism of the present invention may also be identified using any combination of the 11 characteristics set forth above, which provide sufficient information to taxonomically validly describe a microorganism belonging to the same species as R114, R-1512, R1534, and R-1506, with the exception of *Paracoccus* sp. (MBIC3966).

In accordance with the foregoing the present invention provides

(1) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43, in particular an amino acid sequence corresponding to positions 68-97 of SEQ ID NO:43;

(b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45, in particular an amino acid sequence corresponding to positions 1-30 of SEQ ID NO:45;

(c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47, in particular an amino acid sequence corresponding to positions 269-298 of SEQ ID NO:47;

(d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49, in particular an amino acid sequence corresponding to positions 109-138 of SEQ ID NO:49;

(e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51, in particular an amino acid sequence corresponding to positions 198-227 of SEQ ID NO:51;

(f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53, in particular an amino acid sequence corresponding to positions 81-110 of SEQ ID NO:53;

(g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues;

(h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase;

(i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate

isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and

(j) a conservatively modified variant of SEQ ID NO:43, 45, 47, 49, 51 or 53.

(2) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:159;

(c) an amino acid sequence of a fragment of SEQ ID NO: 159, the fragment having the activity of farnesyl diphosphate synthase (FPP synthase);

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 295-1158 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and

(e) a conservatively modified variant of SEQ ID NO:159.

(3) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:160;

(c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS);

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of DXPS;

(e) a conservatively modified variant of SEQ ID NO:160.

(4) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:178;

(c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 178, the fragment having the activity of acetyl-CoA acetyltransferase;

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1-1170 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetyl-CoA acetyltransferase; and

(e) a conservatively modified variant of SEQ ID NO:178.

(5) an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 240 of SEQ ID NO:179;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:179;

(c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 179, the fragment having the activity of acetoacetyl-CoA reductase;

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1258-1980 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetoacetyl-CoA reductase; and

(e) a conservatively modified variant of SEQ ID NO:179.

(6) an isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, variants of SEQ ID NO:42 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having an activity selected from the group consisting of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:42, or the complement of SEQ ID NO:42, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase; in particular

(a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 2622 to 3644 of SEQ ID NO:42, fragments thereof that encode a polypeptide having HMG-CoA reductase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 2622 to 3644 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA reductase activity, more particularly a polynucleotide sequence consisting of nucleotides 2622 to 3644 of SEQ ID NO:42;

(b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 3641 to 4690 of SEQ ID NO:42,

variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having isopentenyl diphosphate isomerase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 3641 to 4690 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having isopentenyl diphosphate isomerase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42.

(c) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 4687 to 5853 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having HMG-CoA synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 4687 to 5853 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having HMG-CoA synthase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;

(d) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 5834 to 6970 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having mevalonate kinase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 5834 to 6970 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having mevalonate kinase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;

(e) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 6970 to 7887 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having phosphomevalonate kinase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 6970 to 7887 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a

polypeptide having phosphomevalonate kinase activity, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42; or

(f) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 7880 to 8878 of SEQ ID NO:42, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:42 that encode a polypeptide having diphosphomevalonate decarboxylase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 7880 to 8878 of SEQ ID NO:42, or a complement thereof, wherein the polynucleotide encodes a polypeptide having diphosphomevalonate decarboxylase activity, more particularly an isolated polynucleotide consisting of nucleotides 7880 to 8878 of SEQ ID NO:42, more particularly a polynucleotide sequence consisting of nucleotides 3641 to 4690 of SEQ ID NO:42;

(7) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:157 that encode a polypeptide having farnesyl diphosphate (FPP) synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or a polypeptide having the activity of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of SEQ ID NO:157, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity, and the activity of XseB, in particular

(a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of a nucleotide sequence spanning positions 59-292 of SEQ ID NO:157, variants thereof containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 59-292 of SEQ ID NO:157 that encode a polypeptide having the function of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 59-292 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having the function of XseB, more particularly an isolated polynucleotide consisting of nucleotides 59 to 292 of SEQ ID NO:157;

(b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 295-1158 of SEQ ID NO:157 that encode a FPP synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 295-1158 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having FPP synthase activity, more particularly an isolated polynucleotide consisting of nucleotides 295 to 1158 of SEQ ID NO:157;

(c) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157, variants of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of the nucleotide sequence spanning positions 1185-1610 of SEQ ID NO:157 that encode a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning positions 1185-1610 of SEQ ID NO:157, or the complement of such a sequence, wherein the polynucleotide encodes a polypeptide having 1-deoxyxylulose-5-phosphate synthase activity, more particularly an isolated polynucleotide consisting of nucleotides 1185 to 1610 of SEQ ID NO:157;

(8) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:177, or the complement of SEQ ID NO:177, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, in particular

(a) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 1 to 1170 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having acetyl-CoA acetyltransferase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 1 to 1170 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetyl-CoA acetyltransferase activity, more particularly an isolated polynucleotide sequence consisting of nucleotides 1-1170 of SEQ ID NO:177;

(b) an isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of nucleotides 1258-1980 of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table (Table 14), fragments of SEQ ID NO:177 that encode a polypeptide having acetoacetyl-CoA reductase activity, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides spanning residues 1258-1980 of SEQ ID NO:177, or a complement thereof, wherein the polynucleotide encodes a polypeptide having acetoacetyl-CoA reductase activity, more particularly an isolated polynucleotide sequence consisting of nucleotides 1258-1980 of SEQ ID NO:177;

(9) an isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and combinations thereof;

(10) an expression vector comprising the polynucleotide sequence of any one of (6) (a) to (6) (f), (7) (a) to (7) (c), (8) (a), (8) (b) or (9), in particular an expression vector wherein the polynucleotide sequence is operably linked to an expression control sequence, e.g. an expression vector further comprising a polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway, more particularly an expression vector wherein the polynucleotide sequence is selected from the group consisting of SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, and combinations thereof which are operably linked to an expression control sequence;

(11) an expression vector selected from the group consisting of pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His–*mvaA*, pDS-His–*idi*, pDS-His-*hcs*, pDS-His–*mvk*, pDS-His–*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-P*crtE*-*crtE*-3,

pBBR-tK-P*crtE-mva*A, pBBR-tK-P*crtE-idi*, pBBR-tK-P*crtE-hcs*, pBBR-tK-P*crtE-mvk*, pBBR-tK-P*crtE-pmk*, pBBR-tK-P*crtE-mvd*, pBBR-K-P*crtE-mvaA-crtE*-3, pDS-His-*phaA*, pBBR-K-P*crtE-crtW*, pBBR-K-P*crtE-crtWZ*, pBBR-K-P*crtE-crtZW*, and combinations thereof, in particular

(a) an expression vector selected from the group consisting of pBBR-K-mev-op16-1 and pBBR-K-mev-op16-2,

(b) an expression vector selected from the group consisting of pBBR-K-Zea4, pBBR-K-Zea4-up, and pBBR-K-Zea4-down;

(c) an expression vector selected from the group consisting of pBBR-K-P*crtE-crtE*-3, pBBR-tK-P*crtE-mva*A, pBBR-tK-P*crtE-idi*, pBBR-tK-P*crtE-hcs*, pBBR-tK-P*crtE-mvk*, pBBR-tK-P*crtE-pmk*, pBBR-tK-P*crtE-mvd*, and combinations thereof;

(d) an expression vector which is pBBR-K-P*crtE-mvaA-crtE*-3;

(e) an expression vector which is pDS-His-*phaA*; or

(f) an expression vector selected from the group consisting of pBBR-K-P*crtE-crtW*, pBBR-K-P*crtE-crtWZ*, and pBBR-K-P*crtE-crtZW*;

(12) a cultured cell comprising the polynucleotide sequence of any one of (6) (a) to (f), (7) (a) to (c), (8) (a), (8) (b) or (9), or an expression vector of (10) or (11), or a progeny of the cell, wherein the cell expresses a polypeptide encoded by the polynucleotide sequence, in particular a cell which is further characterized by a feature selected from

(a) further comprising a polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway, more particularly a cultured cell wherein the polynucleotide sequence that encodes an enzyme in the carotenoid biosynthetic pathway is selected from the group consisting of SEQ ID NOs:180, 182, and 184, or a progeny of the cell, wherein the cell expresses polypeptides encoded by the polynucleotide sequences, and

(b) from being a member of a group selected from yeast, fungus, bacterium and alga, in particular a bacterium selected from the group consisting of *Paracoccus, Flavobacterium*, *Agrobacterium, Alcaligenes, Erwinia, E. coli*, and *B. subtilis*, more particularly *Paracoccus*, more particularly *Paracoccus* selected from the group consisting of R-1506, R-1512, R1534, and R114;

(13) a method of producing a carotenoid comprising culturing a cell of (12) under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence, and isolating the carotenoid from the cell or the medium of the cell;

(14) a method of making a carotenoid-producing cell comprising:

(a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and

(b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence,

in particular a method selected from a method characterized by a feature selected from

(i) the selecting step comprising selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.5-500 times, particularly about 100 times, or at least about 10 times, the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence;

(ii) the cell producing from about 1 mg/L to about 10 g/L of a carotenoid.

(iii) the cell being selected from the group consisting of a yeast, fungus, bacterium, and alga, in particular selected from the group consisting of *Paracoccus, Flavobacterium, Agrobacterium, Alcaligenes, Erwinia, E. coli*, and *B. subtilis*, more particularly from *Paracoccus*;

(iv) the cell in step (a) being a mutant cell, in particular being selected from the group consisting of R114 and R1534, in particular the mutant cell producing about 1.1-1,000 times, in particular about 1.5-500 times, more particularly at least about 100 times more or at least about 10 times more, the level of a carotenoid compared to its non-mutant parent;

(v) the polynucleotide sequence being selected from polynucleotide sequences of (6) (a) to (f), (7) (a) to (c), (8) (a), (8) (b) and (9), in particular wherein the polynucleotide sequence is operably linked to an expression control sequence;

(vi) the polynucleotide sequence being an expression vector of (10) or (11);

(vii) the introducing step being selected from the group consisting of transformation, transduction, transfection, lipofection, electroporation, conjugation, and biolistics.

(viii) the carotenoid being selected from the group consisting of phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof, in particular the carotenoid being zeaxanthin;

(15) a method for engineering a bacterium to produce an isoprenoid compound comprising:

(a) culturing a parent bacterium in a medium under conditions permitting expression of an isoprenoid compound, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid compound than the parent bacterium;

(b) introducing into the mutant bacterium an expression vector comprising a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and

(c) selecting a bacterium that contains the expression vector and produces at least about 1.1 times more of an isoprenoid compound than the mutant in step (a), in particular

(i) a method further comprising introducing a mutation into the mutant bacterium, more particularly a method wherein the mutation causes an effect selected from at least one of the following: inactivating the polyhydroxyalkanoate (PHA) pathway, increasing expression of acetyl-CoA acetyltransferase, increasing expression of farnesyl diphosphate (FPP) synthase, increasing expression of an enzyme in a carotenoid pathway, increasing the expression of an enzyme for converting isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP),

most particularly a method wherein inactivating of the PHA pathway comprises selecting for a mutant bacterium that does not express a polypeptide encoded by *phaB* (nucleotide positions 1258-1980 of SEQ ID NO: 177) or by disrupting expression of the wild type *phaB* gene by homologous recombination using SEQ ID NO:177 or a fragment thereof, or

a method wherein increasing expression of acetyl-CoA acetyltransferase comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence represented by SEQ ID NO:175 or nucleotide positions 1-1170 of SEQ ID NO:177 operably linked to an expression control sequence, or

a method wherein increasing expression of FPP synthase comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence represented by nucleotides 295-1158 of SEQ ID NO:157 operably linked to an expression control sequence, or

a method wherein increasing expression of an enzyme in a carotenoid pathway comprises introducing into the mutant bacterium a vector comprising a polynucleotide sequence selected from the group consisting SEQ ID NOs:180, 182, and 184 operably linked to an expression control sequence;

(b) a method wherein the isoprenoid is isopentenyl diphosphate (IPP).

(c) a method wherein the isoprenoid is a carotenoid, in particular a method wherein the carotenoid is selected from the group consisting of phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone, adonirubin, and combinations thereof;

(d) a method wherein the parent bacterium is a *Paracoccus*, in particular R-1512 or R-1506, or R1534 or R114, in particular wherein the mutant is R114;

(16) a microorganism of the genus *Paracoccus*, which microorganism has the following characteristics:

(i) a sequence similiarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%;

a homology to strain R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C;

a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and

an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534, R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966);

(ii) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes;

an inability to use adonitol, i-erythritol, gentiobiose, β-methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and

an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966); or

(iii) an ability to grow at 40°C;

an ability to grow in a medium having 8% NaCl;

an ability to grow in a medium having a pH of 9.1; and

a yellow-orange colony pigmentation, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

The following examples are provided to further illustrate certain aspects of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

Example 1: Analytical and Biochemical Methods

(a) Analysis of Carotenoids

Sample preparation. A solvent mixture of 1:1 dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) was first prepared. This solvent mixture was stabilized by the addition of butylated hydroxytoluene (BHT, 0.5 g/l solvent mixture). Four milliliters of the stabilized DMSO/THF mixture was added to 0.4 ml of bacterial culture in a disposable 15-ml poly-

propylene centrifuge tube (gives a final dilution factor of 1/11). The tubes were capped and mixed using a Vortex mixer for 10 seconds each. The samples were then put on a Brinkmann Vibramix shaker for 20 minutes. The tubes were centrifuged at room temperature for 4 minutes at 4000 rpm and aliquots of the clear yellow/orange supernatant were transferred into brown glass vials for analysis by High Performance Liquid Chromatography (HPLC).

HPLC. A reversed phase HPLC method was developed for the simultaneous determination of astaxanthin, zeaxanthin, canthaxanthin, β-carotene, and lycopene. The method was also able to separate the main *cis*-isomers of zeaxanthin. Chromatography was performed using an Agilent 1100 HPLC system equipped with a thermostatted autosampler and a diode array detector. The method parameters were as follows:

Column: YMC Carotenoid C30 column, particle size 5 micron
250* 4.6mm I.D., steel
(YMC, Part No. CT99S052546WT)

Guard column: Pelliguard LC-18 cartridge, 20 mm
(SUPELCO, Part No. 59654)

Mobile phase: Methanol (MeOH)/Methyl tert-butyl ether (TBME) gradient

	% MeOH	% TBME
Start	80	20
10 min	65	35
20 min	10	90

Run time: 28 min; Typical column pressure: 90 bar at start; Flow rate: 1.0 ml/min.; Detection: UV at 450 nm; Injection volume: 10 µl; Column temperature: 15°C

Reagents. Methanol and TBME were HPLC grade and were obtained from EM Science and J.T. Baker, respectively. DMSO (Omnisolve) was purchased from EM Science. THF (HPLC solvent) was from Burdick and Jackson.

Calculations. Quantitative analyses were performed with a two level calibration using external standards (provided by Hoffmann-La Roche, Basel, Switzerland). Calculations were based on peak areas.

Selectivity. The selectivity of the method were verified by injecting standard solutions of the relevant carotenoid reference compounds. The target compounds (all-*trans*-carotenoids) were completely separated and showed no interference. Some minor *cis* isomers may coelute, although these potentially interfering isomers are rare and need not be considered in routine analyses. The retention times of the compounds are listed in Table 1.

Table 1. HPLC retention times for carotenoids.

Carotenoid	Retention time (min.)	Carotenoid	Retention time (min.)
Astaxanthin	6.99	Canthaxanthin	9.95
Adonixanthin	7.50	Cryptoxanthin	13.45
15-*cis*-Zeaxanthin	7.80	β-Carotene	17.40
13-*cis*-Zeaxanthin	8.23	Lycopene	21.75
all-*trans*-Zeaxanthin	9.11		

Linearity. 25 Milligrams of all-*trans*-zeaxanthin were dissolved in 50 ml of DMSO/THF mixture (giving a final zeaxanthin concentration 500 µg/ml). A dilution series was prepared (final zeaxanthin concentrations of 250, 100, 50, 10, 5, 1, and 0.1 µg/ml) and analyzed by the HPLC method described above. A linear range was found from 0.1 µg/ml to 250 µg/ml. The correlation coefficient was 0.9998.

Limit of detection.The lower limit of detection for zeaxanthin by this method was determined to be 60 µg/l. A higher injection volume and optimization of the integration parameters made it possible to lower the detection limit to approximately 5 µg/l .

Reproducibility. The retention time for all-*trans*-zeaxanthin was very stable (relative standard deviation (RSD), 0.2 %). The peak area reproducibility, based on ten repetitive analyses of the same culture sample, was determined to be 0.17 % RSD for all *trans*-zeaxanthin and 1.0 % for cryptoxanthin.

(b) Preparation of crude extracts and enzyme assay methods.

Preparation of crude extracts. Crude extracts of *Paracoccus* and *E. coli* were prepared by resuspending washed cell pellets in 1 ml of extraction buffer (buffer used depended on the enzyme being assayed – compositions are specified along with each enzyme assay procedure described below). Cell suspensions were placed in a 2-ml plastic vial and disrupted by agitation with glass beads using a Mini Bead Beater 8 (Biospec Products, Bartlesville, OK, USA). Disruption was performed at 4°C using a medium agitation setting. The disrupted preparations were centrifuged at 21,000 x g for 20 minutes at 4°C to sediment the cell debris, and the supernatants were used directly for enzyme assays.

Protein determinations. Protein concentrations in crude extracts were determined by the method of Bradford [Anal. Biochem. 72:248-254 (1976)] using the Bio-Rad Protein Assay

Reagent (Bio-Rad, Hercules, CA, USA). Bovine serum albumin was used as the reference protein for construction of standard curves.

Acetyl-CoA acetyltransferase assays. Crude extracts were prepared in 150 mM EPPS (*N*-[2-hydroxyethyl] piperizine-*N'*-[3-propanesulfonic acid]) buffer, pH 8.0. Assays were performed in the thiolysis direction according to the method described by Slater et al. [J. Bacteriol. 180:1979-1987 (1998)]. This assay measures the disappearance of acetoacetyl-CoA spectrophotometrically at 304 nm. Reaction mixtures contained 150 mM EPPS buffer (pH 8.0), 50 mM $MgCl_2$, 100 μM CoA, 40 μM acetoacetyl-CoA and crude extract. Reactions were carried out at 30°C and were initiated by addition of crude extract. The disappearance of acetoacetyl-CoA at 304 nm was monitored using a SpectraMAX Plus plate reader (Molecular Devices Corp., Sunnyvale, CA, USA) and a quartz microtiter plate (any standard spectrophotometer can also be used). Activity (expressed as U/mg protein) was calculated using a standard curve constructed with acetoacetyl-CoA (1 unit of activity = 1 μmol acetoacetyl-CoA consumed/min.). The lower limit of detection of Acetyl-CoA acetyltransferase activity was 0.006 U/mg.

HMG-CoA synthase assays. HMG-CoA synthase was assayed according to the method of Honda et al. [Hepatology 27:154-159 (1998)]. In this assay, the formation of HMG-CoA from acetyl-CoA and acetoacetyl-CoA is measured directly by separating the reaction product and substrates by HPLC. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0). Reaction mixtures (0.1 ml) contained 50 mM Tris-HCl buffer (pH 8.0), 0.1 mM EDTA, 20 mM $MgCl_2$, 0.1 mM acetoacetyl-CoA, 0.8 mM acetyl-CoA and crude extract. Reactions were pre-incubated for 2 minutes at 30°C before adding the crude extract. After 5 minutes of reaction at 30°C, the reactions were stopped by adding 0.2 ml of 200 mM tetra-butyl ammonium phosphate (TBAP, dissolved in methanol-water (3:2, final pH was 5.5) and containing 0.2 mM propionyl-CoA as an internal recovery standard). The mixture was then centrifuged for 3 minutes at 21,000 x g at 4°C and subsequently kept on ice until analyzed by reversed phase ion-pair HPLC. HMG-CoA and propionyl-CoA were separated from acetyl-CoA and acetoacetyl-CoA using a Nova-Pak C18 column (3.9 x 150 mm, Waters Corporation, Milford, MA, USA). The injection volume was 20 μl, the mobile phase was 50 mM TBAP dissolved in methanol-water (1:1, final pH was 5.5), and the flow rate was 1.0 ml/min. HMG-CoA and propionyl-CoA were detected by absorbance at 254 nm. HMG-CoA produced in the reaction was quantified by comparison with a standard curve created using authentic HMG-CoA. Activity is defined as U/mg protein. One unit of activity = 1 nmol HMG-CoA produced/min. The lower limit of detection of HMG-CoA synthase was about 1 U/mg.

HMG-CoA reductase assays. Crude extracts were prepared in 25 mM potassium phosphate buffer (pH 7.2) containing 50 mM KCl, 1 mM EDTA and a protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO, USA, catalog #P-2714). Assays were performed according to the method of Takahashi et al. [J. Bacteriol. 181:1256-1263 (1999)]. This assay measures the HMG-CoA dependent oxidation of NADPH spectrophotometrically at 340nm. Reaction mixtures contained 25 mM potassium phosphate buffer (pH 7.2), 50 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 0.3 mM NADPH, 0.3 mM *R,S*-HMG-CoA and crude extract. Reactions were performed at 30°C and were initiated by the addition of HMG-CoA. HMG-CoA-dependent oxidation of NADPH was monitored at 340 nm using a SpectraMAX Plus plate reader (Molecular Devices Corp., Sunnyvale, CA, USA) and a quartz microtiter plate (any standard spectrophotometer may be used). Activity (expressed as U/mg protein) was calculated using a standard curve constructed with NADPH (1 unit of activity = 1 μmol NADPH oxidized/min.). The lower limit of detection of HMG-CoA reductase activity was 0.03 U/mg.

Mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase assays. The preparation of substrates and the assay procedures for mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase have been described in detail by Popják [Methods Enzymol. 15:393-425 (1969)]. For all assays, one unit of enzyme activity is defined as 1μmol of product formed/minute. In addition to these spectrophotometric and radiochromatographic assays, alternate methods, for example using HPLC separation of reaction substrates and products, can be used. The lower limit of detection of mevalonate kinase, phosphomevalonate kinase and mevalonate diphosphate decarboxylase is typically about 0.001 U/mg protein.

IPP isomerase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 7.5). Assays were performed using the method of Spurgeon et al. [Arch. Biochem. Biophys. 230:445-454 (1984)]. This assay is based on the difference in acid-lability of IPP and DMAPP. Reaction mixtures (0.1 ml final volume) contained 50 mM Tris-HCl buffer (pH 7.5), 2 mM dithiothreitol, 5 mM $MgCl_2$, 20 μM [1-^{14}C]-IPP and crude extract. Reactions were carried out at 30°C for 15 minutes and terminated by the addition of 0.3 ml of a mixture of concentrated HCl:methanol (4:1) and an additional incubation at 37°C for 20 min. Hexane (0.9 ml) was added and the tubes were mixed (4 times for 10 seconds using a vortex mixer). After centrifugation (21,000 x g, 5 minutes), 0.6 ml of the hexane layer was transferred to a scintillation vial, scintillation fluid was added, and the radioactivity counted. Activity is expressed as U/mg protein. One unit of activity = 1 pmol [1-^{14}C]-IPP incorporated into acid labile products/min. The lower limit of detection of IPP isomerase activity was 1 U/mg.

FPP synthase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0). The FPP synthase assay procedure was similar to the IPP isomerase assay described above, being based on the difference in acid lability of IPP and FPP (Spurgeon et al., supra). Reaction mixtures (0.1 ml final volume) contained 50 mM Tris-HCl buffer (pH 8.0), 2 mM dithiothreitol, 5 mM $MgCl_2$, 20 μM [1-^{14}C]-IPP, 25 μM GPP (geranyl pyrophosphate) and crude extract. Reactions were carried out at 30°C for 15 minutes and terminated by the addition of 0.3 ml of a mixture of concentrated HCl:methanol (4:1) and an additional incubation at 37°C for 20 minutes. Hexane (0.9 ml) was added and the tubes were mixed (4x, 10 seconds using a vortex mixer). After centrifugation (21,000 x g, 5 minutes), 0.6 ml of the hexane layer was transferred to a scintillation vial, scintillation fluid was added, and the radioactivity counted. Units of enzyme activity, and the lower limit of detection, were the same as defined above for IPP isomerase. In cases where high IPP isomerase activity interferes with measurement of FPP synthase activity, crude extract may be preincubated for 5 minutes in the presence of 5mM iodoacetamide to inhibit IPP isomerase activity.

GGPP synthase assays. Crude extracts were prepared in 50 mM Tris-HCl buffer (pH 8.0) containing 2 mM dithiothreitol. GGPP synthase was assayed according to the procedure of Kuzuguchi et al. [J. Biol. Chem. 274:5888-5894 (1999)]. This assay is based on the same principle as described above for FPP synthase. Reaction mixtures (0.1 ml final volume) contained 50 mM Tris-HCl buffer (pH 8.0), 2 mM dithiothreitol, 5 mM $MgCl_2$, 20 μM [1-^{14}C]-IPP, 25 μM FPP and crude extract. All reaction conditions and subsequent treatment of samples for scintillation counting were identical to those described above for FPP synthase. Treatment of extract with iodoacetamide to inhibit IPP isomerase activity may also be used as above. Units of enzyme activity, and the lower limit of detection, were the same as defined above for IPP isomerase.

Acetoacetyl-CoA reductase assays. Crude extracts are prepared in 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM KCl and 5 mM dithiothreitol. Acetoacetyl-CoA reductase was assayed according to the procedure of Chohan and Copeland [Appl. Environ. Microbiol. 64:2859-2863 (1998)]. This assay measures the acetoacetyl-CoA-dependent oxidation of NADPH spectrophotometrically at 340 nm. Reaction mixtures (1 ml) contain 50 mM Tris-HCl buffer (pH 8.5), 15 mM $MgCl_2$, 250 μM NADPH, and 100 μM acetoacetyl-CoA. Reactions are performed at in a quartz cuvette at 30°C and are initiated by the addition of acetoacetyl-CoA. Activity (expressed as U/mg protein) was calculated using a standard curve constructed with NADPH (1 unit of activity = 1 μmol NADPH oxidized/min). The lower limit of detection of acetoacetyl-CoA reductase activity is about 0.01 U/mg.

Example 2: Taxonomic Reclassification of *Flavobacterium sp.* as *Paracoccus*

This Example describes the taxonomic re-classification of the zeaxanthin-producing bacterium formerly designated *Flavobacterium* sp. strain R-1512 (ATCC 21588) as *Paracoccus* sp. strain R-1512 (ATCC 21588). A comprehensive genomic and biochemical/physiological analysis was performed by the Belgian Coordinated Collections of Microorganisms/Laboratorium voor Microbiologie, Universiteit Gent (BCCM™/LMG), using state-of-the-art methods currently accepted as the scientific standards for bacterial classification. Besides *Paracoccus* sp. strain R-1512, several other bacteria belonging to the genus *Paracoccus* were included in the study (summarized in Table 2).

Table 2. Bacteria used in taxonomic study.

Bacterium	Strain designation	Source or reference
Paracoccus sp.	R-1512 (ATCC 21588)	ATCC (environmental isolate) US 3,891,504
Paracoccus sp.	R1534	mutant derived from R-1512; US 6,087,152
Paracoccus sp.	R114	mutant derived from R-1512; This work
Paracoccus sp.	R-1506	environmental isolate; This work
Paracoccus sp.	MBIC3024	H. Kasai, Kamaishi Institute, Japan
Paracoccus sp.	MBIC3966	H. Kasai, Kamaishi Institute, Japan
Paracoccus sp.	MBIC4017	H. Kasai, Kamaishi Institute, Japan
Paracoccus sp.	MBIC4020	H. Kasai, Kamaishi Institute, Japan
P. marcusii	DSM 11574^T	Harker et al., infra
P. carotinifaciens	E-396^T	Tsubokura et al., infra
P. solventivorans	DSM 6637^T	Siller et. al., Int. J. Syst. Bacteriol. 46:1125-1130 (1996)

Strains R1534 and R114 are mutants derived from strain R-1512 by classical mutagenesis and screening for improved zeaxanthin production. The primary screening was accomplished by selecting the highest color intensity producing colonies. A secondary screening

was accomplished in liquid culture media by the HPLC methods according to Example 1. Strain R-1506 is an independent isolate obtained from the same initial screening of environmental microorganisms that provided strain R-1512. Strains MBIC3024, MBIC3966, MBIC4017 and MBIC4020 were identified as members of the genus *Paracoccus* by the nucleotide sequences of their 16S rDNA genes (DNA sequences were deposited in the public EMBL database, *see* Table 5). *Paracoccus marcusii* DSM 11574^T and *Paracoccus carotinifaciens* E-396^T are recently described type strains of carotenoid-producing bacteria [Harker et al., Int. J. Syst. Bacteriol. 48:543-548 (1998); Tsubokura et al., Int. J. Syst. Bacteriol. 49:277-282 (1999)]. *Paracoccus solventivorans* DSM 6637^T was included as a "control" strain, being a member of the genus *Paracoccus* but distantly related to the other bacteria used.

Preliminary experiments resulted in the following conclusions. Each of the methods set forth herein has a well-recognized ability to define taxonomic relatedness or relative degree of similarity between organisms. The methods and their use for delineating bacterial taxa were described and compared in detail by Van Damme et al., Microbiological Reviews 60:407-438 (1996) and Janssen et al., Microbiology 142:1881-1893 (1996).
(1) Fatty acid analysis of the cell membranes of strains R1534 and R114 showed that the two strains were highly similar and indicated a taxonomic relatedness of these strains to *Paracoccus denitrificans* and *Rhodobacter capsulatus.*
(2) One-dimensional gel electrophoresis of cellular proteins showed a high similarity (*i.e.*, a relatedness at the intra-species level) between R1534 and R114, but the profiles did not justify allocation of these strains to either *R. capsulatus* or *P. denitrificans.*
(3) DNA:DNA hybridization between strain R1534 and *R. capsulatus* $LMG2962^T$ and *P. denitrificans* $LMG4218^T$ confirmed that strain R1534 is neither *R. capsulatus* nor *P. denitrificans.*
(4) Sequencing of 16S rDNA genes from strains R1534 and R114 showed that these organisms belong to the genus *Paracoccus*, but that they represent a new species. The highest degree of sequence similarity was observed with the 16S rDNA gene of *Paracoccus* sp. strains MBIC3966, MBIC4020 and MBIC3024.
(5) DNA fingerprinting of strains R1534 and R-1512 using Amplified Fragment Length Polymorphism (AFLP™) showed high overall similarity of the genomic DNA from the two strains, indicating an infraspecific relatedness (*i.e.* AFLP™ can differentiate between two members of the same species).

In the following sections, the results and conclusions of the present comprehensive taxonomic study of *Paracoccus* sp. strain R-1512 (and its mutant derivatives R1534 and R114) are set forth.

16S rDNA sequencing and phylogenetic study. The bacteria set forth in Table 2 were grown in LMG medium 185 ((TSA) BBL 11768 supplemented where necessary with 1.5% Difco Bacto agar). Genomic DNA was prepared according to the protocol of Niemann et al. [J. Appl. Microbiol. 82:477-484 (1997)]. Genes coding for 16S rDNA were amplified from genomic DNA from strains R-1512, R1534, R114 and R-1506 by polymerase chain reaction (PCR) using the primers shown in Table 3.

Table 3. Primers used for PCR amplification of DNA coding for 16S rDNA in *Paracoccus* sp. strains R-1512, R1534, R114, and R-1506.

Primer name[a]	Sequence (5'→3')	SEQ ID NO	Position[b]
16F27	AGA GTT TGA TCC TGG CTC AG	SEQ ID NO:1	8-27
16F38	CTG GCT CAG GAC/T GAA CGC TG	SEQ ID NO:2	19-38
16R1522	AAG GAG GTG ATC CAG CCG CA	SEQ ID NO:3	1541-1522

[a]F, forward primer; R, reverse primer. Forward primer 16F27 (Synonym: PA) was used for strains R1534 and R-1506, while forward primer 16F38 (Synonym: ARI C/T) was used for strains R-1512 and R114. The reverse primer 16R1522 (Synonym: PH) was used for all strains.

[b]Hybridization position referring to *E. coli* 16S rDNA gene sequence numbering.

The PCR-amplified DNAs were purified using the Qiaquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Complete sequencing was performed using an Applied Biosystems, Inc. 377 DNA Sequencer and the protocols of the manufacturer (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA) using the "ABI PRISM™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (with AmpliTaq® DNA Polymerase, Fs)". The primers used for DNA sequencing are shown in Table 4.

Table 4. Primers used for sequencing PCR-amplified segments of genes coding for 16S rDNA in *Paracoccus* sp. strains R-1512, R1534, R114 and R-1506.

Primer name[a]/-Synonym	Sequence (5'→3')	SEQ ID NO	Position[b]

16F358/*Gamma	CTC CTA CGG GAG GCA GCA GT	SEQ ID NO:4	339-358
16F536/*PD	CAG CAG CCG CGG TAA TAC	SEQ ID NO:5	519-536
16F926/*O	AAC TCA AAG GAA TTG ACG G	SEQ ID NO:6	908-926
16F1112/*3	AGT CCC GCA ACG AGC GCA AC	SEQ ID NO:7	1093-1112
16F1241/*R	GCT ACA CAC GTG CTA CAA TG	SEQ ID NO:8	1222-1241
16R339/Gamma	ACT GCT GCC TCC CGT AGG AG	SEQ ID NO:9	358-339
16R519/PD	GTA TTA CCG CGG CTG CTG	SEQ ID NO:10	536-519
16R1093/3	GTT GCG CTC GTT GCG GGA CT	SEQ ID NO:11	1112-1093

[a]F, forward primer; R, reverse primer.

[b]Hybridization position referring to *E. coli* 16S rDNA gene sequence numbering.

Five forward and three reverse primers were used to obtain a partial overlap of sequences, ensuring highly reliable assembled sequence data. Sequence assembly was performed using the program AutoAssembler (Perkin-Elmer, Applied Biosystems Division, Foster City, CA, USA). Phylogenetic analysis was performed using the software package Gene-Compar™ (v. 2.0, Applied Maths B.V.B.A., Kortrijk, Belgium) after including the consensus sequences (from strains R-1512, R1534, R114 and R-1506) in an alignment of small ribosomal subunit sequences collected from the international nucleotide sequence library EMBL. This alignment was pairwise calculated using an open gap penalty of 100% and a unit gap penalty of 0%. A similarity matrix was created by homology calculation with a gap penalty of 0% and after discarding unknown bases. A resulting tree was constructed using the neighbor-joining method.

The nucleotide sequence of the 16s rDNA gene from *Paracoccus* sp. strain R-1512 is illustrated as SEQ ID NO:12. The distance matrix, presented as the percentage of 16S rDNA sequence similarity, between strain R-1512 and its closest relatives, is shown in Table 5. The sequences from strains R-1512 and its mutant derivatives R1534 and R114 were identical. The sequence from R-1506 differed by only one nucleotide from the sequence from latter strains. This demonstrated strains R-1512 and R-1506 are phylogenetically highly related and likely belong to the same species (confirmed by DNA:DNA hybridization, see below). Comparison of the R-1512 and R-1506 sequences with those publicly available at the EMBL library located R-1512 and R-1506 in the genus *Paracoccus*. However, the sequence similarities observed with all currently taxonomically validly described *Paracoccus* species was <97%, the value generally accepted as the limit for a possible relatedness at the species level [Stackebrandt and Goebel, Int. J. Syst. Bacteriol.

44:846-849 (1994)]. This demonstrated that strains R-1512 (and its mutant derivatives) and R-1506 belong to one or two new *Paracoccus* species. Sequence similarities of >97% (significant for a possible relationship at the species level), were observed between four unnamed *Paracoccus* strains and strains R-1512, R1534, R114 and R-1506, suggesting that one or more of the unnamed (MBIC) strains may relate at the species level to strains R-1512 and R-1506. Based on cluster analysis (phylogenetic tree depicting the phylogenetic relatedness between *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966, and other members of the genus *Paracoccus*), strains R-1512, R1534, R114, R-1506 and four unnamed *Paracoccus* strains (MBIC3024, MBIC3966, MBIC4017 and MBIC4020) were selected for DNA:DNA hybridization experiments to analyze species relatedness.

Table 5. Distance matrix, presented as the percentage of 16S rDNA sequence similarity, between *Paracoccus* sp. strain R-1512 and its closest relatives.

Strain[a]	EMBL Accession number	% Similarity
R-1512	-	100
R1534	-	100
R114	-	100
R-1506	-	99.9
Paracoccus sp. MBIC3966	AB018688	100
Paracoccus sp. MBIC3024	AB008115	98.2
Paracoccus sp. MBIC4020	AB025191	98.1
Paracoccus sp. MBIC4036	AB025192	97.0
Paracoccus sp. MBIC4017	AB025188	96.9
Paracoccus sp. MBIC4019	AB025190	96.8
Paracoccus sp. MBIC4018	AB025189	96.4
Paracoccus marcusii DSM 11574[T]	Y12703	96.2
Paracoccus carotinifaciens E-396[T]	AB006899	96.1
Paracoccus solventivorans DSM 6637[T]	Y07705	95.4
Paracoccus thiocyanaticus THIO11[T]	D32242	95.3
Paracoccus aminophilus JCM 7686[T]	D32239	95.1
Paracoccus alcaliphilus JCM 7364[T]	D32238	95.0

Paracoccus pantotrophicus ATCC 35512^T	Y16933	95.0
Paracoccus denitrificans ATCC 17741^T	Y16927	94.8
Paracoccus versutus IAM 12814^T	D32243	94.7
Paracoccus kocurii JCM 7684^T	D32241	94.6
Paracoccus aminovorans JCM 7685^T	D32240	94.4
Paracoccus alkenifer A901/1^T	Y13827	94.3
Rhodobacter capsulatus ATCC 11166^T	D16428	92.9

[a]Type strains are followed by a ^{T}

DNA:DNA hybridization and determination of G+C content. The bacteria set forth in Table 5 were grown in LMG medium 185. Genomic DNA was prepared according to the protocol of Wilson [In Ausabel et al. (eds.), Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York, 2.4.1-2.4.5 (1987)]. The G+C content of the DNA's was determined by HPLC according to Mesbach et al. [Int. J. Syst. Bacteriol. 39:159-167 (1989)] as modified by Logan et al. [Int. J. Syst. Evol. Microbiol. 50:1741-1753 (2000)]. Reported values are the mean of these measurements on the same DNA sample. DNA:DNA hybridizations were performed using the initial renaturation rate method as described by De Ley et al. [Eur. J. Biochem. 12:133-142 (1970)]. The hybridization temperature was 81.5°C. For this method, an average deviation of +/-5.8% has been reported by Vauterin et al. [Int. J. Syst. Bacteriol. 45:472-489 (1995)]. The G+C content of the bacterial DNA's and the results of the DNA hybridization experiments are summarized in Table 6.

Table 6. G+C content (mol %) of DNA from *Paracoccus* spp. strains and per cent DNA homology between the strains.

Strain	%G+C	% DNA Homology							
R-1512	67.6	100							
R1534	67.7	96	100						
R114	67.5	100	97	100					
R-1506	67.5	94	90	88	100				
MBIC3024	65.4	31	nd[a]	nd	31	100			
MBIC3966	66.9	93	nd	nd	88	32	100		
MBIC4017	67.2	32	nd	nd	31	24	24	100	

MBIC4020	68.4	27	nd	nd	25	25	23	34	100

[a]not determined

Strains R-1512, R1534, R114, R-1506 and MBIC3966 showed a DNA homology of >70% (the generally accepted limit for species delineation [Wayne et al., Int. J. Syst. Bacteriol. 37:463-464 (1987)], and therefore belong to the same species within the genus *Paracoccus.* The G+C content of these five strains varied from 66.9%-67.7%, thus remaining within 1%, characteristic for a well defined species. On the other hand, the low DNA homology between strains MBIC3024, MBIC4017 and MBIC4020 and strains R-1512, R1534, R114, R-1506 and MBIC3966 showed that MBIC3024, MBIC4017 and MBIC4020 each belong to a different genomic species within the genus *Paracoccus.*

DNA fingerprinting using AFLP™. AFLP™ is a PCR-based technique for whole genome DNA fingerprinting via the selective amplification and selective visualization of restriction fragments [Vos et al., Nucleic Acids Research 23:4407-4414 (1995); Janssen et al., supra]. In this analysis, *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966, and *Paracoccus marcusii* DSM 11574^{T} were compared to evaluate infraspecies relatedness. These bacteria were grown in LMG medium 185. Genomic DNA from each of these bacteria was prepared according to the protocol of Wilson (supra). Purified DNA was digested by two restriction enzymes, a 4-base cutter and a 6-base cutter. In this way, a limited number of fragments with two different ends and of suitable size for efficient PCR were obtained. Adaptors (small double-stranded DNA molecules of 15-20 bp) containing one compatible end were ligated to the appropriate "sticky" end of the restriction fragments. Both adaptors are restriction halfsite-specific, and have different sequences. These adaptors serve as binding sites for PCR primers. Here, the restriction enzymes used were *Apa*I (a hexacutter, recognition sequence GGGCC/C) and *Taq*I (a tetracutter, recognition sequence T/GCA). The sequences of the adaptors ligated to the sticky ends generated by cleavage with the restriction enzymes are shown in Table 7 (SEQ ID Nos:13-22). PCR was used for selective amplification of the restriction fragments. The PCR primers specifically annealed with the adaptor ends of the restriction fragments. Because the primers contain, at their 3' end, one so-called "selective base" that extends beyond the restriction site into the fragment, only those restriction fragments that have the appropriate complementary sequence adjacent to the restriction site were amplified. The sequences of the six PCR primer combinations used are also shown in Table 7.

Table 7. Adaptors and PCR primers used for AFLP™ analysis.

	Sequence	SEQ ID NO
Adaptors corresponding to restriction enzyme *ApaI*		
Adaptor 93A03	5'-TCGTAGACTGCGTACAGGCC-3'	SEQ ID NO:13
Adaptor 93A04	3'-CATCTGACGCATGT-5'	SEQ ID NO:14
Adaptors corresponding to restriction enzyme *TaqI*		
Adaptor 94A01	5'-GACGATGAGTCCTGAC-3'	SEQ ID NO:15
Adaptor 94A02	3'-TACTCAGGACTGGC-5'	SEQ ID NO:16
	Sequence	SEQ ID NO
Primer combination 1 (PC A)		
A01	5'GACTGCGTACAGGCCCA3'	SEQ ID NO:17
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 2 (PC B)		
A01	5'GACTGCGTACAGGCCCA3'	SEQ ID NO:17
T02	5'CGATGAGTCCTGACCGAC3'	SEQ ID NO:19
Primer combination 3 (PC D)		
A02	5'GACTGCGTACAGGCCCC3'	SEQ ID NO:20
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 4 (PC I)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T03	5'CGATGAGTCCTGACCGAG3'	SEQ ID NO:22
Primer combination 5 (PC G)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T01	5'CGATGAGTCCTGACCGAA3'	SEQ ID NO:18
Primer combination 6 (PC H)		
A03	5'GACTGCGTACAGGCCCG3'	SEQ ID NO:21
T02	5'CGATGAGTCCTGACCGAC3'	SEQ ID NO:19

Following amplification, the PCR products were separated according to their length on a high resolution polyacrylamide gel using a DNA sequencer (ABI 377). Fragments that contained an adaptor specific for the restriction halfsite created by the 6-bp cutter were

visualized by autoradiography due to the 5'-end labeling of the corresponding primer with ^{32}P. The electrophoretic patterns were scanned and numerically analyzed with Gel-Compar™ 4.2 software (Applied Maths, B.V.B.A., Kortrijk, Belgium) and clustered using the Pearson curve matching coefficient and unweighted pair group averages linking [clustering methods were reviewed by Sneath and Sokal, In: Numerical Taxonomy. Freeman & Son, San Francisco (1973)].

In all six primer combinations (PC A-H, Table 7), the DNA fingerprints of *Paracoccus* sp. strains R-1512, R1534 and R114 were highly similar if not identical. In cases where minor differences were observed, reproducibility was not evaluated. The high similarity or identity among the three strains was expected as strains R1534 and R114 were derived from strain R-1512. With all primer combinations, strains R-1512, R1534 and R114 were clearly discriminated from strains R-1506 and MBIC3966, the latter two strains equally belonging to the new *Paracoccus* species. However, the fingerprints provide no clear indication that strains R-1512, R1534 and R114 are more related to either R-1506 or MBIC3966. Under the conditions used, the five strains of the new species cluster at an average level of about 58% similarity (this value is the mean of the six values of the branching points of the new species in the six AFLP™ experiments (six primer combinations)), and the cluster can clearly be discriminated from the profile of *Paracoccus marcusii* DSM 11574^T, the type strain of a phylogenetically related carotenoid-producing *Paracoccus* species. The mean similarity value of the six branching points for *Paracoccus marcusii* DSM 11574^T and the new species was about 11%.

Fatty acid analysis. The fatty acid composition of the cell membranes of *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966 were compared to the type strains *P. marcusii* DSM 11574^T, *P. carotinifaciens* E-396^T and *P. solventivorans* DSM 6637^T. The bacteria were grown for 24 hours at 28°C in LMG medium 185. The fatty acid compositions were determined by gas chromatography using the commercial system MIDI (Microbial Identification System, Inc., DE , USA). Extraction and analysis of fatty acids was performed according to the recommendations of the MIDI system. Table 8 summarizes the results for all strains tested. For the five strains of the new *Paracoccus* species (R-1512, R1534, R114, R-1506, MBIC3966), the mean profile was calculated. All eight organisms showed a comparable fatty acid composition of their cell membranes, with 18:1 w7c as the major compound. Only minor differences in fatty acid composition were observed between the new *Paracoccus* species and the three type strains.

Utilization of carbon sources for growth. For testing the aerobic utilization of carbon sources, BIOLOG-SF-N Microplate microtiter plates (Biolog Inc., Hayward, CA, USA) containing 95 substrates were used with the exception that the substrate in well E6 was D,L-lactic acid methyl ester instead of the usual sodium salt of D,L-lactic acid. Cells from each of the strains identified in Table 9 were grown for 24 hours at 28°C in LMG medium 12 (Marine Agar, Difco 0979). A cell suspension with a density equivalent to 0.5 McFarland units was prepared in sterile distilled water. From this suspension, 18 drops were transferred into 21 ml of AUX medium (API 20NE, bioMérieux, France) and mixed gently. 0.1 Milliliters of the suspension was transferred to each well of the BIOLOG MicroPlates, and the plates were incubated at 30°C. Wells were visually checked for growth after 48 hours and after 6 days. Also, at 6 days the visual scoring was confirmed by reading the microtiter plates using the BIOLOG plate reader.

The results of the BIOLOG analysis are shown in Table 9. Growth (positive reaction) was determined as increased turbidity compared to the reference well without substrate. A distinction was made between good growth (+), weak growth (±) and no growth (-). Results in parentheses are those obtained after 6 days if different from the results obtained after 48 hours. A question mark indicates an unclear result at 6 days. Of the 95 carbon sources tested, 12 could be used, and 47 could not be used, for growth by all five strains comprising the new *Paracoccus* species (R-1512, R1534, R114, R-1506 and MBIC3966). These five strains gave variable growth responses to the remaining 36 substrates. The new *Paracoccus* species could be distinguished from the two other carotenoid-producing bacteria (*P. marcusii* DSM 11574^T and *P. carotinifaciens* E-396^T) by their inability to use seven carbon sources (adonitol, i-erythritol, gentiobiose, β-methylglucoside, D-sorbitol, xylitol and quinic acid). Two carbon sources that were utilized by all five members of the new *Paracoccus* species (L-asparagine and L-aspartic acid) were not used for growth by *P. marcusii* DSM 11574^T.

Table 8. Fatty acid composition of cell membranes of *Paracoccus* sp. strains R-1512, R1534, R114, R-1506, MBIC3966 and three type strains of other *Paracoccus* species, i.e. *P. marcusii* DSM 11574^T, *P. carotinifaciens* E-396^T and *P. solventivorans* DSM 6637^T

	Mean % for:	% for:		
Name	R-1512, R1534, R114, R-1506 and MBIC3966	DSM 11574^T	E-396^T	DSM 6637^T
10:0 3OH	4.9 ± 1.1	6.2	3.4	3.6

Unnamed 11.799	3.6 ± 0.5	4.9	2.8	3.0
Unnamed 15.275	1.5 ± 0.3	2.9	1.1	ND[a]
16:0	0.3 ± 0.2	ND	0.3	0.7
17:1 w8c	ND	ND	0.6	0.8
17:0	0.1 ± 0.1	ND	0.3	1.3
18:1 w7c	80.5 ± 1.8	80.3	84.0	79.0
	Mean % for:	% for:		
Name	R-1512, R1534, R114, R-1506 and MBIC3966	DSM 11574^T	E-396^T	DSM 6637^T
18:0	3.6 ± 0.4	2.6	5.2	6.6
18:0 3OH	0.6 ± 0.4	ND	ND	ND
19:0	ND	ND	ND	0.7
20:1 w7c	0.8 ± 0.2	ND	0.2	2.0
Summed feature 2	2.7 ± 0.4	3.0	2.1	2.6
Summed feature 3	0.7 ± 0.5	ND	0.2	ND
TOTAL	99.3	99.9	100.2	100.3

[a] ND, not detected

Biochemical tests. Selected biochemical features were tested using the API 20NE strip (bioMérieux, France). Cells from each of the bacterial strains identified in Table 10 were grown for 24 hours at 28°C on LMG medium 12. Cell suspensions were prepared and strips inoculated according to the instructions of the manufacturer. Strips were incubated at 28°C and results determined after 24 and 48 hours. The results are summarized in Table 10. Of the nine features tested, only one (urease activity) gave a variable response among the five strains of the new *Paracoccus* species. These nine tests did not differentiate between the new *Paracoccus* species and *Paracoccus marcusii* DSM 11574^T and *P. carotinifaciens* E-396^T.

Table 9. Utilization of carbon sources for growth by *Paracoccus* spp. strains.

	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574^T	E-396^T	DSM 6637^T

α-Cyclodextrin	-	-	-	-	-	-	-	-
Dextrin	-	-	-	-	-	-	-	-(±)
Glycogen	-	-	-	-	-	-	-	-
Tween 40	-	-	-	-	-	-	-(?)	-
Tween 80	-	-	-	-	-	-	-	-
GalNAc	-	-	-	-	-	-	-	-
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574T	E-396T	DSM 6637T
GlucNAc	-	-	-	-	-	-	-	-(?)
Adonitol	-	-	-	-	-	+	+	-
L-Arabinose	-	-	-	-	-	+	-	+
D-Arabitol	+	+	+	+	±(+)	+	+	-
Cellobiose	±(+)	±(+)	-(?)	-(+)	-(±)	+	+	-(+)
i-Erythritol	-	-	-	-	-	+	+	-
D-Fructose	+	+	+	+	-	+	+	+
L-Fucose	-	-	-	-	-	-	+	-
D-Galactose	+	+	+	±(+)	±(+)	+	+	-(±)
Gentiobiose	-	-	-	-	-	+	+	-(±)
α-D-Glucose	+	+	+	±(+)	-(+)	+	±(+)	+
m-Inositol	+	+	+	-(+)	-(+)	+	-(±)	-
α-Lactose	+	±(+)	-(+)	-(+)	-(+)	+	+	±(+)
Lactulose	-(±)	-(+)	-(+)	-(±)	-	+	+	-(+)
Maltose	+	+	-(+)	-(+)	-(±)	+	+	-(+)
D-Mannitol	+	+	+	+	-(+)	+	+	-(±)
D-Mannose	+	+	+	+	-(±)	+	+	-(+)
D-Melibiose	+	+	+	-(+)	-(+)	+	+	-(?)
β-Methylgluc	-	-	-	-	-	+	+	+

D-Psicose	-(+)	±(+)	±(+)	-	-(+)	-	±	-
D-Raffinose	-	-	-	-	-	-(+)	+	-
L-Rhamnose	-	-	-	-	-	-	-	-(?)
D-Sorbitol	-	-	-	-	-	+	+	-
Sucrose	+	+	±(+)	-(+)	-	+	+	+
D-Trehalose	+	+	-(+)	-(+)	-(+)	+	+	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574^T	E-396^T	DSM 6637^T
Turanose	-(+)	-(+)	-	-	-	+	+	+
Xylitol	-	-	-	-	-	+	+	-
Methylpyruvate	±	-	±	-(?)	±	-	+	-(±)
MMSucc	±(+)	±	-(±)	-(+)	-(±)	-(+)	+	-
Acetic acid	-	-	±	-	-	-	-	+
Cis-aconitic acid	-	±	±	-	-	±	-	-
Citric acid	-	±	±	-	-	±	-	-
Formic acid	-	-	-	-	-	-	-	-
GalAlactone	-(±)	-(±)	-(±)	-	-	-	-(±)	-(?)
GalacturonicA	-	-	-	-	-	-(+)	-(±)	-
D-Gluconic acid	+	+	+	-(±)	-(±)	+	+	+
GlucosaminicA	-	-	-	-	-	-	-	-
GlucuronicA	±	+	+	-(±)	-	±(+)	-	-
AHBA	-(±)	-	-(±)	-	-(+)	-	-	-
BHBA	+	+	+	-(±)	±	-(+)	+	+
GHBA	-	-	-	-	-	-	-	-
PHPAA	-	-	-	-	-	-	-	-(+)
Itaconic acid	-	-	-	-	-	-	-	-

AKBA	-	-	-	-	-	-	-	-(±)
AKGA	-	-	-	-(±)	-(?)	-(±)	-(+)	-(±)
AKVA	-	-	-	-	-	-	-	-
LAME	-	-	-	-	-	-	-	-
Malonic acid	-	-	-	-	-	-	-	-
Propionic acid	-	±	±	-	-	±	+	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574^T	E-396^T	DSM 6637^T
Quinic acid	-	-	-	-	-	+	+	-
SaccA	-(+)	±	-	-(±)	-	-	-	-
Sebacic acid	-(+)	-(+)	-(+)	-(+)	-(±)	-	-(+)	-
Succinic acid	-	-	-	-	-	-(+)	±	-(?)
BromosuccA	-	-	-	-	-	±	-	-
Succinamic acid	-	-	-	-	-	-(+)	-(+)	-
Glucuronamide	-	-	-	-	-(±)	-	-	-
Alaninamide	-	-	-	-	-	-(+)	+	-
D-Alanine	-	-	-(+)	-	-	-	-	-
L-Alanine	+	+	+	+	-	-(+)	+	+
L-Alanyl-glycine	-(+)	-	-(+)	-	-	-	-(+)	-(?)
L-Asparagine	+	+	±(+)	+	±(+)	-	+	-
L-Aspartic acid	+	+	±(+)	-(+)	-(+)	-	+	-
L-Glutamic acid	+	+	+	+	±(+)	-(+)	+	-(+)
GAA	-	-	-	-(±)	-	-	-	-
GGA	-(?)	-	-	-(?)	-	-	-(±)	-
L-Histidine	-	-	-	-	-	-(?)	-	+
HydPro	-	-	-	-	-	-	-	+
L-Leucine	-(±)	-(+)	-(+)	-(+)	-	-(+)	-(?)	-(+)

L-Ornithine	-	-(+)	±(+)	-(±)	-	-	-(+)	-
L-Phenylalanine	-	-	-	-	-	-	-	-
L-Proline	+	+	+	+	-	-(+)	+	+
PyroGluA	+	+	+	+	±(+)	-(+)	+	-
D-Serine	-	-	-	-	-	-	-	-
L-Serine	±	±(+)	-(+)	-(±)	-(+)	-(+)	-(+)	+
	R1512	R1534	R114	R1506	MBIC 3966	DSM 11574^T	E-396^T	DSM 6637^T
L-Threonine	-	-	-	-	-	-(+)	-	-
D,L-Carnitine	-	-	-	-	-	-	-	-
GABA	-	-	-	-	-(+)	-	-(+)	-(+)
Urocanic acid	-	-	-	-	-	-	-	-(+)
Inosine	-	-(±)	-	-	-	-(±)	-(+)	-(+)
Uridine	-	-	-	-	-	-(±)	-(+)	-
Thymidine	-	-	-	-	-	-(±)	-(±)	-
PEA	-	-	-	-	-	-	-	-
Putresceine	-	-	-	-	-	-	-	-
2-Aminoethanol	-	-	-	-	-	-	-	-
2,3-Butanediol	-	-	-	-	-	-	-	-
Glycerol	+	+	+	-(+)	-	+	+	-
GlycP	-	-	-	-	-(±)	-	-	-
Gluc-1-P	-	-	-	-	-	-	-(±)	-
Gluc-6-P	-	-	-	-	-	-	-	-

GalNAc: N-Acetyl-D-galactosamine; GlucNAc: N-Acetyl-D-glucosamine; β-Methylgluc: β-Methylglucoside; MMSucc: Mono-methylsuccinate; GalAlactone: D-Galactonic acid lactone; GalacturonicA: D-Galacturonic acid; GlucosaminicA: D-Glucosaminic acid; GlucuronicA: D-Glucuronic acid; AHBA: α-Hydroxybutyric acid; BHBA: β-Hydroxy-butyric acid; GHBA: γ-Hydroxybutyric acid; PHPAA: p-Hydroxyphenylacetic acid; AKBA:

α-Ketobutyric acid; AKGA: α-Ketoglutaric acid; AKVA: α-Ketovaleric acid; LAME: D,L-Lactic acid methyl ester; SaccA: D-Saccharic acid; BromosuccA: Bromosuccinic acid; GAA: Glycyl-L-aspartic acid; GGA: Glycyl-L-glutamic acid; HydPro: Hydroxy-L-proline; PyroGluA: L-Pyroglutamic acid; GABA: γ-Aminobutyric acid; PEA: Phenylethylamine; GlycP: D,L-α-Glycerolphosphate; Gluc-1-P: Glucose-1-phosphate; Gluc-6-P: Glucose-6-phosphate

Table 10. Biochemical features of *Paracoccus* spp. strains: 12 = R1512; 34 = R1534; 14 = R114, 06 = R1506; 66 = MBIC3966; 74 = DSM 11574^T, 96 = E-396^T, 37 = DSM 6637^T

	12	34	14	06	66	74	96	37
Reduction nitrate to nitrite	-	-	-	-	-	-	-	+
Reduction nitrate to nitrogen	-	-	-	-	-	-	-	+
Indole from tryptophan	-	-	-	-	-	-	-	-
Fermentation of glucose	-	-	-	-	-	-	-	-
Arginine hydrolase	-	-	-	-	-	-	-	-
Urease	S / + 5	-	-	S / + 5	+	-	-	-
Esculine hydrolysis[a]	weak	S / + 5	S / + 5	+	+	+	+	+
Gelatine hydrolysis[b]	-	-	-	-	-	-	-	-
β-Galactosidase	+	+	+	+	+	+	+	-

[a]: β-glucosidase; [b]: protease; S / + 5: Slow + 5 days

Physiological tests. Several physiological and morphological tests were performed on the five strains of the new *Paracoccus* species, along with *Paracoccus marcusii* DSM 11574^T, *Paracoccus carotinifaciens* E-396^T and *Paracoccus solventivorans* DSM 6637^T. The methods used for each test were as follows.

Temperature range for growth. Cells were grown for 24 hours at 28°C on LMG medium 12. A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred onto the agar surface of LMG medium 12. One drop was diluted by streaking, the other 2 drops were left undisturbed. The plates were incubated under aerobic conditions at 10°C, 25°C, 30°C, 33°C, 37°C and 40°C, and checked for growth after 24 hours, 48 hours and 5 days. Growth was determined as visual growth (confluent in the drops and as colonies in the streaks with

diluted inoculum) compared to the growth at 30°C (i.e., the "control"). Scoring was done (vs. the control plate) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth (±), poor growth (±), and no growth (-). Results in parentheses are those observed in the streaks if different from the confluent growth in the undisturbed drops.

Salt tolerance. Cells were grown for 24 hours at 28°C on LMG medium 12. A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred onto the agar surface of LMG medium 12 supplemented with NaCl to reach final concentrations of 3%, 6% and 8%. One drop was diluted by streaking, the other 2 drops were left undisturbed. The plates were incubated under aerobic conditions at 28°C and checked for growth after 24 hours, 48 hours and 5 days. Growth was determined as visual growth (confluent in the drops and as colonies in the streaks with diluted inoculum) compared to the growth without added NaCl (control). Scoring was done (vs. the control plate) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth (±), poor growth (±), and no growth (-). Results in parentheses are those observed in the streaks if different from the confluent growth in the undisturbed drops.

pH Range for growth. Cells were grown for 24 hours at 28°C in LMG medium 12. A cell suspension with a density of between 1-2 McFarland units was prepared in sterile distilled water. From this suspension, 3 drops were transferred into tubes containing 10 ml liquid LMG medium 12 with modified pH, giving final pH values after autoclaving of pH 6.1, pH 6.3, pH 7.0, pH 7.7, pH 8.1 and pH 9.1. The liquid cultures were incubated aerobically (with shaking) at 28°C. Growth was checked at 24 hours, 48 hours, 3 days and 6 days. Growth was determined as increased turbidity (measured as % transmission using the BIOLOG turbidimeter) compared to growth at pH 7.0 (control). Scoring was done (vs. the control) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth (±), poor growth (±), and no growth (-).

Starch hydrolysis. Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and transferred as one streak onto the agar surface of LMG medium 12 supplemented with 0.2% soluble starch. Plates were then incubated under aerobic conditions at 28°C. When the strains had developed good growth (after 48 hours), the plate was flooded with lugol solution (0.5% I_2 and 1% KI in distilled water). Hydrolysis was determined as a clear zone alongside the growth (in contrast to the blue color of the agar where starch was not hydrolyzed).

Denitrification. Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and stabbed into tubes containing semi-solid (0.1% agar) LMG medium 12 supplemented with 1% KNO_3. The plates were incubated at 28°C for 5 days. Denitrification (N_2 from nitrate) was determined as gas formation alongside the stab.

Growth under anaerobiosis without electron acceptor added. Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and streaked onto the agar surface of LMG medium 12. The agar plates were incubated under anaerobic conditions (ca. 10% CO_2 + ca. 90% N_2) at 30°C. Plates were checked for growth after 24 hours and after 5 days. Growth was determined visually and compared to the aerobic (control) condition. Scoring was done (vs. the control) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth (±), poor growth ($\pm$), and no growth (-).

Growth under anaerobic conditions with glucose added (fermentation). Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and stabbed into tubes containing the basal agar medium of Hugh and Leifson [J. Bacteriol. 66:24-26 (1953)]. Paraffin oil was added to the top of the medium, and the tubes were incubated at 30°C. Tubes were checked for growth and acid formation after 48 hours and after 5 days. Growth was determined visually. Scoring was done as follows; good growth (+), poor growth ($\pm$), and no growth (-).

Growth under anaerobic conditions with KNO_3 as electron acceptor. Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A loopful of cells was taken from the plate and streaked onto the agar surface of LMG medium 12 supplemented with 0.1% KNO_3. The plates were incubated under anaerobic conditions (ca. 10% CO_2 + ca. 90% N_2) at 30°C, and checked for growth after 3 days. Growth was determined as visual growth compared to the aerobic (control) condition. Scoring was done (vs. the control) as follows; better growth (++), good (equivalent to the control) growth (+), weaker growth (±), poor growth ($\pm$), and no growth (-).

Catalase and oxidase reactions. Cells were grown for 24 hours at 28°C on LMG medium 12 plates. A positive result for catalase activity was the production of gas bubbles after suspending a colony in one drop of 10% H_2O_2. A positive result for oxidase activity was the development of a purple-red color after rubbing a colony on filter paper soaked with 1% tetramethylparaphenylene.

Colony pigmentation. Cells were grown for 5 days at 28°C on LMG medium 12. Color of colonies was observed visually.

Cell morphology and motility. Cells were grown for 24 hours at 28°C on LMG medium 12. Cell suspensions were made in sterile saline. Cell morphology and motility were observed microscopically using an Olympus light microscope equipped with phase contrast optics (magnification 1000x).

The results of the physiological and morphological tests are summarized in Table 11. The five strains of the new *Paracoccus* species responded essentially identically in all physiological and morphological tests performed. The tests that gave identical responses for all five strains of the new *Paracoccus* species and that allowed discrimination of these organisms from *Paracoccus marcusii* DSM 11574^T and/or *Paracoccus carotinifaciens* E-396^T were: growth at 40°C, growth with 8% NaCl, growth at pH 9.1, and colony pigmentation.

Zeaxanthin production in strains R-1512, R1534, R114 and R-1506 strains. Strains R-1512, R1534, R114, and R-1506 were grown in ME medium, which contains (per liter distilled water): 5 g glucose, 10 g yeast extract, 10 g tryptone, 30 g NaCl and 5 g $MgSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 7.2 with 5N NaOH before sterilizing by autoclaving. All cultures (25-ml volume in 250-ml baffled Erlenmeyer flasks with plastic caps) were grown at 28°C with shaking at 200 rpm. Seed cultures were inoculated from frozen glycerolized stocks and grown overnight. Aliquots were transferred to the experimental flasks to achieve an initial optical density at 660 nm (OD_{660}) of 0.16. Cultures were then grown at 28°C with shaking at 200 rpm. Growth was monitored throughout the cultivation and at 6, 10 (or 15 for strain R114), and 24 hours, an aliquot of the culture was removed for analysis of carotenoids by the method described in Example 1.

The doubling times of strains R-1512, R1534 and R-1506 under these conditions were 0.85 hours, 1.15 hours and 1.05 hours, respectively. Strain R114 reproducibly exhibited a biphasic growth profile; the doubling time of strain R114 in the initial phase was 1.4 hours while the doubling time in the second phase was 3.2 hours.

Table 12 shows the zeaxanthin production and Specific Formation (zeaxanthin production normalized to OD_{660}) by the *Paracoccus* sp. strains in ME medium. The data are averages of four independent experiments, and within each experiment each strain was tested in duplicate flasks. The improved zeaxanthin production in the classically-derived mutant strains R1534 and R114 compared to the parental strain R-1512 is clearly shown. Zea-

xanthin production by strain R-1506 was approximately the same as strain R-1512. No other carotenoids were detected in any of the cultures.

Table 11. Physiological characteristics of *Paracoccus* spp. strains: 12 = R1512; 34 = R1534; 14 = R114, 06 = R1506; 66 = MBIC3966; 74 = DSM 11574[T], 96 = E-396[T], 37 = DSM 6637[T]

Time [days]	12	34	14	06	66	74	96	37
	Growth at 10°C							
1	-	-	-	-	-	-	-	-
5	± (±)	± (±)	± (-)	± (±)	± (-)	± (±)	± (±)	± (±)
	Growth at 25°C							
1	+	+	+	+	+ (±)	+ (±)	+ (±)	+ (-)
5	+	+	+	+	+	+	+	+
	Growth at 30°C							
1	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
	Growth at 33°C							
1	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+
	Growth at 37°C							
1	+	+	+ (±)	+	+	± (-)	± (-)	+
5	+	+	+	+	+	± (-)	± (±)	+
	Growth at 40°C							
1	+	+ (±)	+ (-)	+ (±)	± (-)	-	-	+ (*)
5	+	+ (±)	+ (-)	+	+ (-)	-	-	+ (*)
	Growth with 3% NaCl							
1	+	+	+	+	+	+	+	±
5	+	+	+	+	+	+	+	+
	Growth with 6% NaCl							
1	+ (±)	± (±)	± (±)	+	± (±)	± (-)	± (-)	-
5	+	+	+	+	+ (*)	+ (±)	+ (±)	-

Time [days]	12	34	14	06	66	74	96	37
	Growth with 8% NaCl							
1	+ (±)	± (±)	± (-)	+(±)	± (±)	-	-	-
5	+	+	+	+	+ (*)	± (-)	± (-)	-
	Growth at pH 6.1							
1	+	+	+	+	-	-	-	-
6	+	+	+	+	+	+	+	+
	Growth at pH 6.3							
1	+	+	+	+	+	±	+	±
6	+	+	+	+	+	+	+	+
	Growth at pH 7.0							
1	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+
	Growth at pH 7.7							
1	+	+	+	+	+	±	±	±
6	+	+	+	+	+	+	+	+
	Growth at pH 8.1							
1	+	+	+	+	+	-	-	±
6	+	+	+	+	+	+	+	+
	Growth at pH 9.1							
1	±	+	-	-	+	-	-	-
6	+	+	+	+	+	-	+	+
	Starch hydrolysis							
	-	-	-	-	-	-	-	-
	Denitrification							
	-	-	-	-	-	-	-	+
	Growth in anaerobiosis without electron acceptor added							
	-	-	-	-	-	-	-	-

	12	34	14	06	66	74	96	37
	Growth in anaerobiosis with glucose added (fermentation)							
	-	-	-	-	-	-	-	±
	Growth in anaerobiosis with KNO_3 added							
	-	-	-	-	-	-	-	-
	Catalase reaction							
	+	+	+	+	+	+	+	+
	Oxidase reaction							
	+	+	+	+	+	+	+	+
	Gram stain							
	-	-	-	-	-	-	-	-
	Motility							
	-	-	-	-	-	-	-	-
	Colony pigmentation							
	Y - O	Y - O	Y - O	Y - O	Y - O	O - P	O -P	P Y
	Cell morphology							
	S to C	S to C	S to C	C	S to C	S	S	S
	Cell dimensions (µm)							
	0.8 x 1.2	0.8 x 1.2	0.8 x 1.2	0.9 x 1.1	0.8 x 1.2 to 1.5	0.8 x 1.5 to 2.0	0.9 x 2.0 to 2.5	0.8 x 1.5 to 2.0

Y -O: yellow-orange; O - P: orange-pink; P Y: pale yellow; S to C: short rod to coccoid; S: short rod; C: coccoid

Table 12. Zeaxanthin production by *Paracoccus* sp. strains R-1512, R1534, R114 and R-1506.

		Zeaxanthin (mg/l)		Specific Formation (mg zeaxanthin/OD_{660})	
Strain	Time (h)	Average	Standard Deviation	Average	Standard Deviation
R-1512	6	0.23	0.10	0.10	0.04
	10	2.05	0.70	0.25	0.08
	24	3.78	0.59	0.38	0.06
R1534	6	0.75	0.10	0.26	0.02
	10	3.45	0.57	0.43	0.07
	24	9.13	0.97	0.95	0.06
R114	6	0.65	0.17	0.86	0.24
	15	7.53	1.12	1.13	0.21
	24	19.7	1.82	2.68	0.20
R-1506	6	0.13	0.06	0.07	0.01
	10	1.35	0.31	0.19	0.04
	24	3.55	0.68	0.38	0.07

Example 3: IPP Biosynthesis via the Mevalonate Pathway in the Zeaxanthin-Producing *Paracoccus* sp. strain R114.

In order to determine the biosynthetic origin (*i.e.*, the mevalonate or DXP pathway) of isoprenoid precursors in *Paracoccus* sp. strain R114, a "retrobiosynthesis" approach [Eisenreich and Bacher, In: Setlow (ed.), Genetic Engineering, Principles and Methods, Kluwer Academic/Plenum Publishers, New York 22:121-153 (2000)] was taken. This predictive approach for data analysis permits the unequivocal assessment of glucose catabolism from the analysis of a single down-stream natural product. In the present work, this involved growth of the bacterium in media containing various binary mixtures of unlabeled glucose and specific ^{13}C-labeled glucoses, followed by purification of the zeaxanthin produced and analysis of the labeling patterns by NMR spectroscopy. Details of the methods used and the experimental results are given below.

<u>Growth of *Paracoccus* sp. strain R114 for ^{13}C labeling experiments</u>. Unlabelled D-glucose monohydrate was purchased from Fluka (Milwaukee, WI, USA). [U-$^{13}C_6$]-D-Glucose was purchased from Isotec (Miamisburg, OH, USA), while [1-$^{13}C_1$] D-glucose, [2-$^{13}C_1$] D-glucose and [6-$^{13}C_1$] D-glucose were from Cambridge Isotope Laboratories (Andover, MA, USA). Yeast extract and peptone (from casein, pancreatically digested) were purchased

from EM Science (Gibbstown, NJ, USA). All other salts and solvents were analytical grade and were purchased from standard chemicals suppliers.

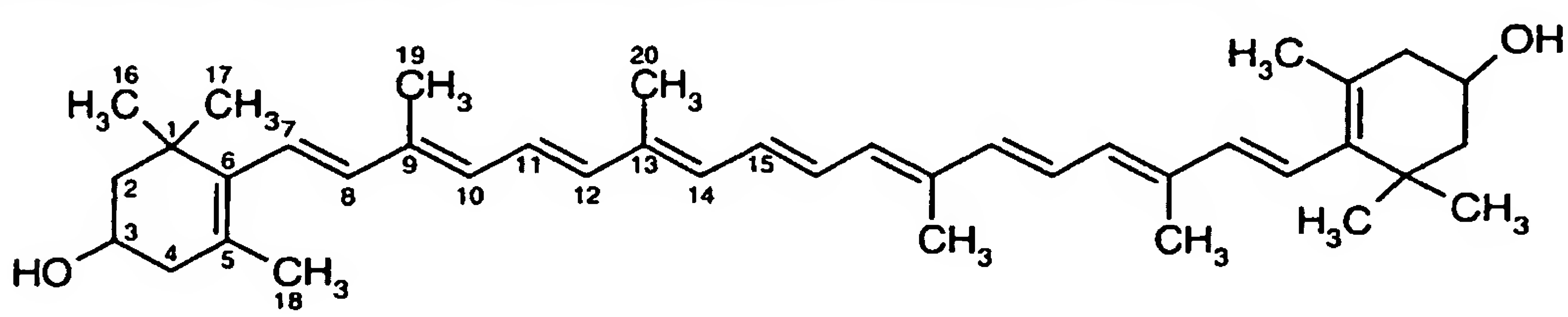
All cultures were initiated from frozen cell suspensions (cell density of 12 OD_{660} units, 25% glycerol, stored at -70°C). One ml of thawed cell suspension was used to inoculate pre-cultures (500-ml baffled shake flasks) containing 100 ml of 362F/2medium having the following composition: 30 g/l D-glucose, 10 g/l yeast extract, 10 g/l peptone, 5 g/l NaCl, 2.5 g/l $MgSO_4 \cdot 7H_2O$, 0.75 g/l $(NH_4)_2HPO_4$, 0.625 g/l K_2HPO_4, 187.5 mg/l $CaCl_2 \cdot 2H_2O$, 0.2 g/l $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, 15 mg/l $ZnSO_4 \cdot 7H_2O$, 12.5 mg/l $FeCl_3 \cdot 6H_2O$, 5 mg/l $MnSO_4 \cdot H_2O$, 0.5 mg/l $NiSO_4 \cdot 6H_2O$, 15 mg/l Na-EDTA and 9.375 µl/l HCl (37% stock solution). The initial pH of the medium was 7.2.

The pre-culture was incubated at 28°C with shaking at 200 rpm for 24 h, after which time the OD_{660} was about 22 absorbance units. The main cultures were grown in Bioflo 3000 bioreactors (New Brunswick Scientific, Edison, NJ, USA) containing 362F/2 medium containing the following composition: 30 g/l total D-glucose (see below for ratios of ^{13}C-labeled:unlabeled glucose), 20 g/l yeast extract, 10 g/l peptone, 10 g/l NaCl, 5 g/l $MgSO_4 \cdot 7H_2O$, 1.5 g/l $(NH_4)_2HPO_4$, 1.25 g/l K_2HPO_4, 0.4 g/l $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, 375 mg/l $CaCl_2 \cdot 2H_2O$, 30 mg/l $ZnSO_4 \cdot 7H_2O$, 25 mg/l $FeCl_3 \cdot 6H_2O$, 10 mg/l $MnSO_4 \cdot H_2O$, 1 mg/l $NiSO_4 \cdot 6H_2O$, 30 mg/l Na-EDTA and 18.75 µl/l HCl (37% stock solution). The amounts of each ^{13}C-labeled glucose used (expressed as a percentage of the total 30 g/l glucose in the medium) in four separate experiments were: Condition 1, 4% [U-$^{13}C_6$] D-glucose; Condition 2, 50% [1-$^{13}C_1$] D-glucose; Condition 3, 25% [2-$^{13}C_1$] D-glucose + 1% [U-$^{13}C_6$] D-glucose; Condition 4, 25% [6-$^{13}C_1$] D-glucose + 1% [U-$^{13}C_6$] D-glucose. A control with only unlabeled glucose was also included. For Conditions 1 and 2 (and the unlabeled control), the culture volume was 2 l, while the culture volume for Conditions 3 and 4 was 1 l. The bioreactors were inoculated with pre-culture (20 ml/l initial volume) and cultivation proceeded for 22-24 hours, at which time no glucose was left in the medium. Cultivation conditions were: 28°C, pH 7.2 (controlled with 25% H_3PO_4 and 28% NH_4OH), dissolved oxygen controlled (in a cascade with agitation) at a minimum of 40%, agitation rate and aeration rate 300 rpm (minimum) and 1 vvm, respectively.

<u>Purification of zeaxanthin</u>. At the end of the cultivations, the cultures were cooled down to 15°C. Five hundred ml of absolute ethanol was added per liter of culture and stirring was continued at 100 rpm for 20 min. The treated culture was centrifuged for 20 min. at 5000 x g, and the supernatant was discarded. The wet pellet was then extracted with 5 volumes of THF for 20 min. with stirring. The extracted mixture was centrifuged, the

supernatant saved and the resulting pellet extracted a second time with 1 volume THF under the same conditions and again centrifuged. The supernatants (extracts) were combined and concentrated to 50 ml by rotary evaporation. Five milliliters of hexane were added to the concentrated THF solution. After mixing, the system formed an emulsion that could be separated by centrifugation. The aqueous phase was collected, diluted with an equal volume of saturated NaCl solution and re-extracted with dichloromethane. The dichloromethane phase was collected and combined with the THF/hexane phase. The mixture of organic extracts was concentrated again in a rotary evaporator to remove dichloromethane. The solution was then applied to a silica gel column and eluted with a mixture of n-hexane and ether (1:1). A small light yellow band eluted first and was discarded. The main zeaxanthin product eluted in a broad band that moved slowly in the column. About 2 liters of solvent was needed to elute the main band completely. The eluate was collected in a round-bottomed flask and the solvent was removed by rotary evaporation at 40°C. The residue was dissolved in a small amount of dichloroethane at 40°C and the solution was then allowed to cool slowly. Hexane was added to the mixture dropwise until a turbidity was observed. The crystallization was complete within 48 hours at 4°C. The crystals were collected on a paper filter, washed with cold methanol and dried under vacuum.

NMR studies. Zeaxanthin was analyzed by NMR spectroscopy. For reference, the chemical structure of zeaxanthin is illustrated in the following formula



^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500.13 MHz and 125.6 MHz, respectively, with a Bruker DRX 500 spectrometer. Acquisition and processing parameters for one-dimensional experiments and two-dimensional INADEQUATE experiments were according to standard Bruker software (XWINNMR). The solvent was deuterated chloroform. The chemical shifts were referenced to solvent signals.

^{13}C NMR spectra of the isotope labeled zeaxanthin samples and of the zeaxanthin sample at natural ^{13}C abundance were recorded under the same experimental conditions. Integrals were determined for every ^{13}C NMR signal, and the signal integral for each respective carbon atom in the labeled compound was referenced to that of the natural abundance material, thus affording relative ^{13}C abundances for each position in the labeled

molecular species. The relative abundances were then converted into absolute abundances from ^{13}C coupling satellites in the ^{1}H NMR signal of H-18 at 1.71 ppm. In the ^{13}C NMR spectrum of the multiply-labeled zeaxanthin sample each satellite was integrated separately. The integral of each respective satellite pair was then referenced to the total signal integral of a given carbon atom. Zeaxanthin comprises a total of eight isoprenoid moieties (2 DMAPP units and 6 IPP units); only 20 ^{13}C NMR signals are observed due to chemical shift degeneracy.

In the experiment with the mixture of [U-$^{13}C_6$] glucose and unlabeled glucose (1:7.5; w/w), all carbon atoms of zeaxanthin were labeled and showed satellites due to $^{13}C^{13}C$ couplings (Table 13). The signals of 4 carbon atoms have intense satellites due to $^{13}C^{13}C$ couplings (61.2 ± 0.6 % in the global NMR signal intensity of a given atom, Table 13). The signal accounting for the methyl atoms C-17/C-17' displayed only weak ^{13}C-coupled satellites at a relative intensity of 6%. The central signals represent material derived from unlabeled glucose. The signals showed no evidence of long-range coupling. Carbon connectivity was easily gleaned from $^{13}C^{13}C$ coupling constants (Table 13) and from two-dimensional INADEQUATE experiments.

Three of the carbon atoms acquired label from [6-$^{13}C_1$] glucose. The other two carbons were labeled from [2-$^{13}C_1$] glucose. No significant amounts of label were contributed to zeaxanthin by [1-$^{13}C_1$] glucose.

The ^{13}C abundance for all non-isochronous carbon atoms was determined by comparison with spectra of unlabeled zeaxanthin and by evaluation of the $^{1}H^{13}C$ coupling satellites in ^{1}H NMR spectra (Table 13). The fraction of jointly transferred carbon atom pairs in the experiment with [U-$^{13}C_6$] glucose was determined by integration of the coupling satellites.

The labeling patterns of the IPP building block can be reconstructed accurately as shown by the standard deviations found for the reconstructed IPP precursor. The reconstructed labeling patterns of DMAPP and IPP were identical within the experimental limits.

Table 13. NMR results for ^{13}C labeled zeaxanthin produced by *Paracoccus* sp. strain R114 supplied with ^{13}C labeled glucoses.

Position	δ - ^{13}C ppm	J_{CC}, Hz	^{13}C-labeled glucose precursor				
			[1-^{13}C]-	[2-^{13}C]-	[6-^{13}C]-	[U-$^{13}C_6$]glucose	
	134.08	44.2 (18, 18')	% ^{13}C	% ^{13}C	% ^{13}C	% ^{13}C	% ^{13}C^{13}C
1, 1′	37.13	36.0 (16,16')	1.10	10.71	2.22	3.47	61.2
2, 2′	48.46	35.8 (3,3')	1.20	2.58	10.27	3.65	61.1
3, 3′	65.10	35.8 (2,2')	1.12	12.47	2.38	3.64	60.4
4, 4′	42.57	37.1	1.27	2.59	10.63	3.89	8.4
5, 5′	126.17	44.2 (18, 18')	1.14	12.45	3.19	3.68	61.1
6, 6′	137.77	56.4 (7,7')	1.30	2.15	9.98	3.60	60.4
7, 7′	125.59	56.2 (6,6')	1.12	10.11	2.82	4.09	61.4
8, 8′	138.50	71.6, 55.7	1.28	2.24	9.95	3.92	4.3, 5.0
9, 9′	135.69	43.1 (19,19')	1.12	9.53	2.95	3.84	61.7
10, 10′	131.31	59.7 (11,11')	1.21	3.18	9.61	3.80	61.1
11, 11′	124.93	59.7 (10,10')	1.10	8.79	2.70	4.02	61.0
12, 12′	137.57	70.5	1.20	2.01	8.80	3.59	5.1
13, 13′	136.48	43.1 (20,20')	1.12	9.86	3.59	3.93	61.4
14, 14′	132.60	60.4 (15,15')	1.21	2.83	10.51	3.77	59.5
15, 15′	130.08	60.4 (14,14')	1.12	9.18	3.33	4.02	61.2
16, 16′	30.26	36.3 (1,1')	1.27	3.19	12.31	3.91	62.0
17, 17′	28.73	34.9 (1,1')	1.30	3.43	12.31	3.88	6.0
18, 18′	21.62	44.2 (5,5')	1.27	3.01	11.66	3.70	62.0
19, 19′	12.82	43.1 (9,9')	1.29	3.12	11.64	3.86	62.3
20, 20′	12.75	42.9 (13,13')	1.33	3.21	11.99	3.75	62.1

The experimental labeling patterns determined above can be compared with various predictions, taking into account not only the mevalonate pathway vs. the DXP pathway for isoprenoid biosynthesis, but also different pathways of glucose metabolism. Eubacteria typically utilize glucose primarily via the glycolytic pathway or via the Entner-Doudoroff pathway. Glycolysis generates two triose phosphate molecules from glucose. The C-1 and C-6 of glucose are both diverted to the 3-position of the triose phosphates produced during glycolysis. On the other hand, in the Entner-Doudoroff pathway, glucose is converted to a mixture of glyceraldehyde 3-phosphate and pyruvate. The C-1 of glucose is

exclusively diverted to C-1 of pyruvate, and the C-6 of glucose is exclusively diverted to C-3 of glyceraldehyde 3-phosphate.

Intermediates and products of the glycolytic and Entner-Doudoroff pathways serve as starting material for both isoprenoid biosynthetic pathways. With regard to the mevalonate pathway, pyruvate as well as triose phosphate can be converted to the precursor acetyl-CoA. Glucose catabolism via the glycolytic pathway diverts label from C-1 as well as C-6 of glucose to the methyl group of acetyl-CoA. Glucose catabolism via the Entner-Doudoroff pathway results in loss of C-1 from glucose during the transformation of pyruvate to acetyl-CoA.

The experimentally observed enrichment and $^{13}C^{13}C$ coupling patterns of the zeaxanthin produced by *Paracoccus* sp. strain R114 were in perfect agreement with the labeling pattern required for zeaxanthin biosynthesis by the combination of the Entner-Doudoroff pathway and the mevalonate pathway. If both the glycolytic and Entner-Doudoroff pathways had been simultaneously operative under the experimental conditions used, at least some label from [1-$^{13}C_1$] glucose should have been contributed to the zeaxanthin. Furthermore, the mevalonate pathway can at best contribute blocks of two carbon atoms to terpenoids, while in the DXP pathway three carbon units can be delivered to isoprenoids via triose phosphate precursors. Although such three-carbon blocks become separated by the rearrangement involved in the DXP pathway, blocks of three labeled carbon atoms can still be recognized via long-range coupling. Corresponding ^{13}C-^{13}C long-range couplings have been observed in the biosynthesis of the carotenoid lutein from [2,3,4,5-$^{13}C_4$] 1-deoxy-D-xylulose by cultured plant cells (*Cantharantus roseus*) [Arigoni et al., Proc. Nat. Acad. Sci. 94:10600-10605 (1997)]. No such long-range coupling was observed in the present experiments with zeaxanthin produced by *Paracoccus* sp. strain R114.

It should be noted that while the results presented here confirm isoprenoid production in *Paracoccus* sp. strain R114 via the mevalonate pathway, and indicate that, under the growth conditions used, there was little or no glucose metabolism via glycolysis, they do not rule out the possibility of some metabolism of glucose via the pentose phosphate pathway in addition to the Entner-Doudoroff pathway. Quantitative determination of glucose metabolism via the latter two pathways could be obtained by analysis of labeling patterns of pyruvate-derived amino acids (as was done for *Paracoccus denitrificans* [Dunstan et al., Biomedical and Environ. Mass Spectrometry 19:369-381 (1990)].

Example 4: Cloning and Sequencing of the Genes Encoding IPP Isomerase and the Enzymes of the Mevalonate Pathway from *Paracoccus* sp. strain R114.

Culture conditions. *Paracoccus* sp. strain R114 was grown at 28°C in F-medium (10 g/l tryptone, 10 g/l yeast extract, 30 g/l NaCl, 10 g/l D-glucose, 5 g/l $MgSO_4{\cdot}7H_2O$, pH 7.0) or in the pre-culture medium described in Example 3 above. Liquid cultures were grown in a rotary shaker at 200 rpm.

Isolation of genomic DNA. A 600-ml culture of *Paracoccus* sp. strain R114 was centrifuged for 10 minutes at 10,000 x g at 4°C and the pellet was washed once with 200 ml lysis buffer (0.1M NaCl, 50mM EDTA, 10mM Tris-HCl, pH 7.5) and once with 100 ml lysis buffer. The final pellet was resuspended in 20 ml lysis buffer containing 50 mg lysozyme and 1 mg RNase A (DNase free). After incubation for 15 minutes at 37°C, 1.5 ml of 20% sodium N-lauroyl-sarcosinate and 2.25 mg of proteinase K were added. After incubation at 50°C for 30-60 minutes, the lysate was extracted with one volume of buffer-saturated phenol, pH 7.5-7.8 (LifeTechnologies, Rockville, MD, USA) by gentle but thorough mixing. The emulsion was centrifuged for 20 minutes at 30,000 x g and the aqueous phase was re-extracted with phenol. The phases were separated as before and the aqueous phase was extracted twice with one volume phenol:chloroform (1:1). At this step centrifugation for 20 minutes at 3,200 x g in a swinging bucket rotor was sufficient to obtain satisfactory phase separation. After a final extraction with one volume of chloroform, 0.1 volume 3M sodium-acetate (pH 5.2) was added and the solution was overlaid with 2 volumes ice-cold ethanol. The precipitated DNA was spooled with a glass-rod, soaked in 70% ethanol for 5 minutes, rinsed with chloroform and then air dried for 5-10 minutes. The DNA was re-suspended overnight in 5 ml TE (10mM Tris-HCl, pH 7.5, 1mM EDTA). Since the solution was yellow due to traces of zeaxanthin, the organic extractions and the spooling were repeated as above to obtain a clear preparation.

Isolation of λ-DNA: The Qiagen® Lambda Kit (Qiagen, Hilden, Germany) was used following the manufacturer's instructions.

Polymerase chain reaction (PCR): Oligonucleotides were purchased from LifeTechnologies (Rockville, MD, USA). PCR was performed in a GeneAmp® PCR system 9700 (PE Applied Biosystems, Foster City, CA, USA) using the GC-rich PCR system (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturers instructions. Typically, the $MgCl_2$ concentration used was 1.5mM and the resolution solution was added to 1M final concentration.

DNA Labeling and detection: The PCR DIG Probe Synthesis Kit and the DIG Luminescent Detection Kit were used for DNA labeling and detection, respectively (both obtained from Roche Molecular Biochemicals, Mannheim, Germany)

DNA sequencing: Sequencing reactions were performed using the BigDye® DNA sequencing kit (PE Applied Biosystems, Foster City, CA, USA) according to the manufacturers instructions. Sequencing reactions were purified on DyeEx™ spin columns (Qiagen, Hilden, Germany) and fragment separation and detection was done with an ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA).

λ-library: A custom made library with partially *Sau*3AI digested *Paracoccus* sp. strain R114 DNA in lambda FIX® II was purchased from Stratagene (La Jolla, CA, USA).

Cloning, sequencing and characterization of the mevalonate pathway gene cluster from *Paracoccus* sp. strain R114. One of the enzymes of the mevalonate pathway, mevalonate diphosphate decarboxylase, contains highly conserved regions spanning several amino acids. Three such regions were chosen from an alignment of all available eubacterial mevalonate diphosphate decarboxylases and oligonucleotides were designed using the preferred codon usage found in the carotenoid gene cluster of *Paracoccus* sp. strain R1534 (Table 14).

The oligonucleotides designed from two homology regions are shown in Table 15. To reduce the degree of degeneracy, sets of oligonucleotides were designed from each peptide. For instance, oligonucleotides mvd-103a-d differ only in the third nucleotide from the 3' end, each accounting for one possible codon for glycine (GGA, although rarely used, was included because of the close proximity to the 3' end). Alternate amino acids were accounted for by designing oligonucleotides to both residues, *e.g.* oligonucleotides mvd-101a and mvd-101b are specific for leucine or isoleucine, respectively, in the second position of peptide 1 (Table 15). PCR with oligonucleotides mvd-101 and mvd-104 or mvd-106, using *Paracoccus* sp. strain 114 DNA as template, gave a product of the expected size. The PCR product was cloned in the vector pCR®2.1-TOPO (Invitrogen, Carlsbad, CA, USA) and sequenced. The cloned fragment was used as a probe for a Southern analysis of *Paracoccus* sp. strain R114 DNA and was found to hybridize to a *Bam*HI-*Sal*I fragment of about 950 bp. *Paracoccus* sp. strain R114 DNA was cut with *Bam*HI and *Sal*I and the fragments were separated by agarose gel electrophoresis. The region around 950 bp was isolated and cloned in the vector pUC19. This partial library was then screened using the *mvd*-PCR fragment as a probe and the insert of a positive clone was sequenced. In parallel, a λ-library prepared from *Paracoccus* sp. strain R114 DNA was screened using the *mvd*-PCR fragment as a probe. DNA was isolated from two positive λ-clones and cut with *Bam*HI and *Sal*I or *Eco*RI and *Sal*I. A number of the restriction fragments were isolated and cloned in the vector pUC19. Several of the fragments contained sequences homologous to genes encoding proteins of the mevalonate pathway. The clones connecting these individual sequences were obtained by PCR with primers derived from the sequences of

the cloned restriction fragments using the DNA of the λ-clones as template. The assembled sequence from all fragments (SEQ ID NO:42, 44, 46, 48, 50, and 52) and the sequences of the encoded proteins are shown in the Sequence Listing (SEQ ID Nos:43, 45, 47, 49, 51, and 53). Due to a limitation of the PatentIn Program, operons with overlapping genes cannot be shown as a single sequence. Thus, for each gene in the mevalonate operon, the entire nucleotide sequence of the operon is repeated for each gene. Accordingly, SEQ ID Nos:42, 44, 46, 48, 50, and 52 are identical. For purposes of the present invention, we use SEQ ID NO:42 to refer to the nucleotide sequence of the mevalonate operon.

The arrangement of the mevalonate pathway genes in the *Paracoccus* sp. strain R114 is unique when compared to known mevalonate gene clusters of other bacteria. Besides *Paracoccus* sp. strain R114, only *Borrelia burgdorferi* and *Streptomyces* sp. strain CL190 (Takagi et al., supra) have all mevalonate genes in a single operon (Wilding et al., supra). In *Streptococcus pyrogenes* all mevalonate genes are clustered in a single locus but they are grouped in two operons. All other species have two loci with the two kinases and the mevalonate diphosphate decarboxylase grouped in one operon and the HMG-CoA synthase and the HMG-CoA reductase on a second locus, either forming an operon (in *Streptococcus pneumoniae*) or as separate transcription units. All species except the members of *Staphylococcus* have an additional gene linked with the mevalonate cluster, which was recently identified as an IPP isomerase (*idi* gene in *Streptomyces* sp. strain CL190) (Kaneda et al., supra). The two *Enterococcus* species and *Staphylococcus haemolyticus* have an acetyl-CoA acetyltransferase gene linked with the HMG-CoA reductase gene. In the *Enterococcus* species the latter two genes are fused.

Table 14: Codon usage in *Paracoccus* sp. strain R1534 carotenoid (*crt*) genes

Amino acid	Codon	Number used	% Used
A – Ala	GCT	3	1.4
	GCC	96	46.2
	GCA	15	7.2
	GCG	94	45.2
C – Cys	TGT	0	0.0
	TGC	15	100.0
D – Asp	GAT	46	38.0
	GAC	75	62.0
E – Glu	GAA	17	25.4
	GAG	50	74.6
F – Phe	TTT	3	5.6
	TTC	51	94.4
G – Gly	GGT	16	10.8
	GGC	87	58.8
	GGA	5	3.4
	GGG	40	27.0
H – His	CAT	30	56.6
	CAC	23	43.4
I – Ile	ATT	5	6.4
	ATC	72	92.3
	ATA	1	1.3
K – Lys	AAA	4	14.3
	AAG	24	85.7
L – Leu	TTA	0	0.0
	TTG	5	2.9
	CTT	15	8.7
	CTC	11	6.4
	CTA	1	0.6
	CTG	140	81.4
M – Met	ATG	49	100.0
N – Asn	AAT	4	20.0
	AAC	16	80.0

Amino acid	Codon	Number used	% Used
P – Pro	CCT	2	2.3
	CCC	41	47.7
	CCA	3	3.5
	CCG	40	46.5
Q – Gln	CAA	6	11.3
	CAG	47	88.7
R – Arg	CGT	11	7.3
	CGC	103	68.2
	CGA	2	1.3
	CGG	26	17.2
	AGA	2	1.3
	AGG	7	4.6
S – Ser	TCT	1	1.1
	TCC	17	19.5
	TCA	0	0.0
	TCG	39	44.8
	AGT	2	2.3
	AGC	28	32.2
T – Thr	ACT	2	2.7
	ACC	36	48.9
	ACA	4	5.3
	ACG	33	44.0
V – Val	GTT	6	5.7
	GTC	61	57.5
	GTA	1	0.9
	GTG	38	35.8
W – Trp	TGG	27	100.0
Y – Tyr	TAT	28	62.2
	TAC	17	37.8

Table 15: Oligonucleotides designed from two conserved bacterial Mvd peptides.

		SEQ[a]
Peptide 1	AlaLeuIleLysTyrTrpGlyLys Ile[2]	23
Nucleotide sequence[1]	CCSCTGATCAARTAYTGGGGBAARATC	24
Oligonucleotide mvd-101a (5' – 3')	GCSCTGATCAARTAYTGGGG	25
Oligonucleotide mvd-101b (5' – 3')	GCSATCATCAARTAYTGGGG	26
Oligonucleotide mvd-103a (5' – 3')	ATCAARTAYTGGGGTAA	27
Oligonucleotide mvd-103b (5' – 3')	ATCAARTAYTGGGGCAA	28
Oligonucleotide mvd-103c (5' – 3')	ATCAARTAYTGGGGGAA	29
Oligonucleotide mvd-103d (5' – 3')	ATCAARTAYTGGGGAAA	30
Peptide 2	ThrMetAspAlaGlyProAsnVal Gln[2]	31
Nucleotide sequence[1] (5'-3')	ACSATGGAYGCSGGBCCSAAYGTS CAR	32
Complement (3'-5')	TGSTACCTRCGSCCVGGSTTRCAS GTY	33
Oligonucleotide mvd-104a (3' – 5')	TGGTACCTACGSCCVGG	34
Oligonucleotide mvd-104b (3' – 5')	TGGTACCTGCGSCCVGG	35
Oligonucleotide mvd-104c (3' – 5')	TGCTACCTACGSCCVGG	36
Oligonucleotide mvd-104d (3' – 5')	TGCTACCTGCGSCCVGG	37
Oligonucleotide mvd-106a (3' – 5')	TACCTACGSCCVGGSTTRCA	38
Oligonucleotide mvd-106b (3' – 5')	TACCTGCGSCCVGGSTTRCA	39
Oligonucleotide mvd-106c (3' – 5')	TACCTACGSCCVGGSGTYCA	40
Oligonucleotide mvd-106d (3' – 5')	TACCTGCGSCCVGGSGTYCA	41

[a]: SEQ ID NO:

[1] using the preferred codons of *Paracoccus* sp. strain R1534, see table 1

[2] alternate amino acid present in some enzyme

S = C or G; R = A or G; Y = C or T; B = C or G or T; V = A or C or G

The genes of the mevalonate operon from *Paracoccus* sp. strain R114 were identified by homology of the gene products to proteins in general databases. An amino acid alignment of the HMG-CoA reductase from *Paracoccus* sp. strain R114 (SEQ ID NO:43) was performed with bacterial class I HMG-CoA reductases of *Streptomyces* sp. Strain CL190 (SEQ

ID NO:54), *S. griseolosporeus* (SEQ ID NO:55), and *Streptomyces* sp. strain KO-3899 (SEQ ID NO:56). EMBL/GenBank/DDBJ database accession numbers are q9z9n4 for *Streptomyces* sp. strain CL190, q9znh1 for *S. griseolosporeus* and q9znh0 for *Streptomyces* sp. strain KO-3899. There are two classes of HMG-CoA reductases [Bochar et al., Mol. Genet. Metab. 66:122-127 (1999); Boucher et al., Mol. Microbiol. 37:703-716 (2000)]. Eubacterial HMG-CoA reductases are generally of class II, whereas class I enzymes are found in eukaryotes and archaea. The *Streptomyces* and the *Paracoccus* HMG-CoA reductases together with the enzyme from *Vibrio cholerae* are the only eubacterial HMG-CoA reductases of class I known so far.

An amino acid alignment of isopentenyl diphosphate isomerase (IPP isomerase) (*idi*) from *Paracoccus* sp. strain R114 (SEQ ID NO:45) was performed with close homologs found in the EMBL database, i.e. *Erwinia herbicola* (Q01335) (SEQ ID NO:57), *Borrelia burgdorferi* (O51627) (SEQ ID NO:58), *Synechocystis* sp. PCC 6803 (P74287) (SEQ ID NO:59), *Streptomyces* sp. CL190 (Q9KWG2) (SEQ ID NO:60), *Streptomyces griseolosporeus* (Q9KWF6) (SEQ ID NO:61), *Sulfolobus solfataricus* (P95997) (SEQ ID NO:62), *Rickettsia prowazekii* (Q9ZD90) (SEQ ID NO:63), *Deinococcus radiodurans* (Q9RVE2) (SEQ ID NO:64), *Aeropyrum pernix* (Q9YB30) (SEQ ID NO:65), *Halobacterium* sp. NRC-1 (O54623) (SEQ ID NO:66), *Archaeoglobus fulgidus* (O27997) (SEQ ID NO:67), *Pyrococcus abyssi* (Q9UZS9) (SEQ ID NO:68), *Pyrococcus horikoshii* (O58893) (SEQ ID NO:69), *Methanobacterium thermoautotrophicum* (O26154) (SEQ ID NO:70), *Methanococcus jannaschii* (Q58272) (SEQ ID NO:71), *Thermoplasma acidophilum* (CAC11250) (SEQ ID NO:72) and *Leishmania major* (Q9NDJ5) (SEQ ID NO:73). EMBL/GenBank/DDBJ database accession numbers are given after the organism's name in parentheses. The first nine sequences are from eubacteria and the next eight sequences are from archaea. Interestingly, one eukaryotic species, the protozoan parasite *Leishmania major* (SEQ ID NO:73), also has a protein that is highly homologous. This is unexpected because other eukaryotes have a different *idi*, designated type 1 (Kaneda et al., supra). A conserved hypothetical protein from *Bacillus subtilis*, YpgA, also has substantial homology but is considerably smaller than the type 2 *idi*'s.

An amino acid alignment of bacterial HMG-CoA synthase from *Paracoccus* sp. strain R114 (SEQ ID NO:47) was performed with close homologs found in the EMBL database, i.e. *Streptococcus pneumoniae* (AAG02453) (SEQ ID NO:74), *Streptococcus pyrogenes* (AAG02448) (SEQ ID NO:75), *Entereococcus faecalis* (AAG02438) (SEQ ID NO:76), *Enterococcus faecium* (AAG02443) (SEQ ID NO:77), *Staphylococcus haemolyticus* (AAG02427) (SEQ ID NO:78), *Staphylococcus epidermis* (AAG02433) (SEQ ID NO:79),

Staphylococcus aureus (AAG02422) (SEQ ID NO:80), *Staphylococcus carnosus* (Q9ZB67) (SEQ ID NO:81), *Streptomyces* sp. CL190 (Q9KWG1) (SEQ ID NO:82), *Streptomyces griseolosporeus* (Q9KWF5) (SEQ ID NO:83) and *Borrelia burgdorferi* (051626) (SEQ ID NO:84). EMBL/GenBank/DDBJ database accession numbers are given after each organism's name in parentheses. The first 43 amino acids of the sequence from *Streptomyces griseolosporeus* are missing in the database version.

An amino acid alignment of bacterial mevalonate diphosphate decarboxylase from *Paracoccus* sp. strain R114 (SEQ ID NO:53) was performed with the orthologous proteins from other bacteria, i.e. *Streptococcus pneumoniae* (AAG02456) (SEQ ID NO:85), *Streptococcus pyrogenes* (AAG02451) (SEQ ID NO:86), *Entereococcus faecalis* (AAG02441) (SEQ ID NO:87), *Enterococcus faecium* (AAG02446) (SEQ ID NO:88), *Staphylococcus haemolyticus* (AAG02431) (SEQ ID NO:89), *Staphylococcus epidermis* (AAG02436) (SEQ ID NO:90), *Staphylococcus aureus* (AAG02425) (SEQ ID NO:91), *Streptomyces* sp. CL190 (Q9KWG4) (SEQ ID NO:92), *Streptomyces griseolosporeus* (Q9KWF8) (SEQ ID NO:93) and *Borrelia burgdorferi* (051629) (SEQ ID NO:94). EMBL/GenBank/DDBJ database accession numbers are given after each organism's name in parentheses.

Two proteins from *Myxococcus xanthus*, Tac and Taf (database accession numbers q9xb06 and q9xb03, respectively) and a protein from *B. subtilis*, PksG, a putative polyketide biosynthesis protein (database accession number p40830), have substantial homology to the *Paracoccus* sp. strain R114 HMG-CoA synthase. The homology between the *Paracoccus* sp. strain R114 HMG-CoA synthase and the Tac and Taf proteins of the *M. xanthus* is greater than the homology between the HMG-CoA synthases from *Paracoccus* sp. strain R114 and eukaryotes. The bacterial HMG-CoA synthases and the bacterial mevalonate diphosphate decarboxylases share substantial homology with their eukaryotic orthologs. Archaeal HMG-CoA synthases form a more distantly related group of enzymes (Wilding et al., supra) and no mevalonate diphosphate decarboxylase orthologs are found in archaea [Smit and Mushegian, Genome Res. 10:1468-1484 (2000)].

Alignments of the mevalonate kinase (Mvk) (SEQ ID NO:49) and the phosphomevalonate kinase (Pmk) (SEQ ID NO:51) from *Paracoccus* sp. strain R114 were performed to the orthologous proteins from other bacteria, i.e. *Streptococcus pneumoniae* (AAG02455) (SEQ ID NO:95), *Streptococcus pyrogenes* (AAG02450) (SEQ ID NO:96), *Entereococcus faecalis* (AAG02440) (SEQ ID NO:97), *Enterococcus faecium* (AAG02445) (SEQ ID NO:98), *Staphylococcus haemolyticus* (AAG02430) (SEQ ID NO:99), *Staphylococcus epidermis* (AAG02435) (SEQ ID NO:100), *Staphylococcus aureus* (AAG02424) (SEQ ID NO:101), *Streptomyces* sp. CL190 (Q9KWG5) (SEQ ID NO:102), *Streptomyces griseolosporeus*

(Q9KWF9) (SEQ ID NO:103) and *Borrelia burgdorferi* (051631) (SEQ ID NO:104)(Mvk); and *Streptococcus pneumoniae* (AAG02457) (SEQ ID NO:105), *Streptococcus pyrogenes* (AAG02452) (SEQ ID NO:106), *Entereococcus faecalis* (AAG02442) (SEQ ID NO:107), *Enterococcus faecium* (AAG02447) (SEQ ID NO:108), *Staphylococcus haemolyticus* (AAG02432) (SEQ ID NO:109), *Staphylococcus epidermis* (AAG02437) (SEQ ID NO:110), *Staphylococcus aureus* (AAG02426) (SEQ ID NO:111), *Streptomyces* sp. CL190 (Q9KWG3) (SEQ ID NO:112), *Streptomyces griseolosporeus* (Q9KWF7) (SEQ ID NO:113) and *Borrelia burgdorferi* (051630) (SEQ ID NO:114) (Pmk). EMBL/GenBank/DDBJ database accession numbers are given after each organism's name in parentheses.

There is much less homology among the bacterial kinases than among the bacterial orthologs of the other enzymes of the mevalonate pathway. The mevalonate kinase from *Paracoccus* sp. strain R114 (SEQ ID NO:49) has a 37 amino acid insert in the amino-terminal region, which is lacking in other mevalonate kinases. Together with the bacterial Mvk's some archaeal enzymes, *e.g.* from *Archaeoglobus fulgidus*, *Methanobacterium thermoautotrophicum* and *Pyrococcus abyssi*, are among the best homologues to the Mvk from *Paracoccus* sp. strain R114. The homology among bacterial phosphomevalonate kinases is even weaker than the homology among the bacterial mevalonate kinases. The proteins with the best homologies to the Pmk from *Paracoccus* sp. strain R114 (SEQ ID NO:51) are Mvk's from archaea, *e.g. Aeropyrum pernix*, *Pyrococcus horikoshii*, *M. thermoautotrophicum*, *P. abyssi* and *A. fulgidus*. Since no Pmk's are found in archaea (Smit and Mushegian, supra), this suggests that the same kinase might perform both phosphorylations.

Example 5: Over-expression of the Mevalonate Pathway Genes and the *idi* Gene from *Paracoccus* sp. strain R114 in *E. coli*

Cloning and expression of the mevalonate operon in *E. coli*. A λ clone, designated clone 16, from the *Paracoccus* sp. strain R114 λ library (see Example 4) was used as a template for PCR amplification of the entire mevalonate operon. Primers Mevop-2020 and Mevop-9027 (Table 16) were used for PCR.

Table 16. Primers used for amplification of mevalonate operon from *Paracoccus* sp. strain R114.

Primer	Sequence (5'→3')
Mevop-2020	GGGCAAGCTTGTCCACGGCACGACCAAGCA (SEQ ID NO:115)
Mevop-9027	CGTAATCCGCGGCCGCGTTTCCAGCGCGTC (SEQ ID NO:116)

The resulting PCR product was cloned in TOPO-XL (Invitrogen, Carlsbad, CA, USA), resulting in plasmid TOPO-XL-mev-op16. The insert carrying the mevalonate operon was excised with *Hin*dIII and *Sac*I and cloned in the *Hin*dIII-*Sac*I cut vector pBBR1MCS2 [Kovach et al., Gene 166:175-176 (1995)], resulting in plasmid pBBR-K-mev-op16. Plasmid pBBR-K-mev-op16 was used to transform electroporation-competent *E. coli* strain TG1 [Stratagene, La Jolla, CA; Sambrook et al., In: Nolan, C. (ed.), Molecular Cloning: A Laboratory Manual (Second Edition), p. A.12 (1989)]. Two representative positive transformants (*E. coli* TG1/ pBBR-K-mev-op16-1 and *E. coli* TG1/ pBBR-K-mev-op16-2) were grown in Luria Broth (LB, GibcoBRL, Life Technologies) containing 50 mg/l kanamycin and tested for HMG-CoA reductase activity (encoded by the *Paracoccus* sp. strain R114 *mvaA* gene) using the methods described in Example 1. *E. coli* does not possess a gene coding for the enzyme HMG-CoA reductase, hence the lack of detectable activity. The crude extracts of both representative transformants of *E. coli* TG1/ pBBR-K-mev-op16 had easily measurable HMG-CoA reductase activity, demonstrating the heterologous expression of the cloned *mvaA* gene.

Table 17. HMG-CoA reductase activity in crude extracts of *E. coli* TG1 cells carrying the cloned mevalonate gene cluster from *Paracoccus* sp. strain R114.

Strain	HMG-CoA reductase activity (U/mg)
E. coli TG1	Not detected[a]
E. coli TG1/ pBBR-K-mev-op16-1	0.25
E. coli TG1/ pBBR-K-mev-op16-2	0.78

[a]Less than 0.03 U/mg

<u>Cloning and expression of the *idi* gene and the individual mevalonate pathway genes from *Paracoccus* sp. strain R114 in *E. coli*</u>. The coding regions of the mevalonate operon genes from *Paracoccus* sp. strain R114 were amplified by PCR using the primers shown in Table 18. The primers were designed such that the ATG start codons constituted the second half of an *Nde*I site (cleavage recognition site CATATG), and *Bam*HI sites (GGATCC) were introduced immediately after the stop codons. All PCR products were cloned in the pCR®2.1-TOPO vector. The names of the resulting vectors are listed in Table 19. Except for the mevalonate kinase gene, all genes contained restriction sites for *Bam*HI, *Nde*I or *Eco*RI, which had to be eliminated in order to facilitate later cloning steps. The sites were eliminated by introducing silent mutations using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and the oligonucleotides shown in Table 20. The mutagenized coding regions were excised from the TOPO-plasmids with *Bam*HI and *Nde*I and ligated with the *Bam*HI-*Nde*I cleaved expression vectors pDS-His and pDS.

These expression vectors were derived from pDSNdeHis, which is described in Example 2 of EP 821,063. The plasmid pDS-His was constructed from pDSNdeHis by deleting a 857 bp *Nhe*I and *Xba*I fragment carrying a silent chloramphenicol acetyltransferase gene. The plasmid pDS was constructed from pDS-His by replacing a small *Eco*RI-*Bam*HI fragment with the annealed primers S/D-1 (5' AATTAAAGGAGGGTTTCATATGAATTCG) (SEQ ID NO:117) and S/D-2 (5' GATCCGAATTCATATGAAACCCTCCTTT) (SEQ ID NO:118).

Table 18: Oligonucleotides for the cloning of the mevalonate operon genes.

Gene	Forward primer		Reverse primer	
	Name	Sequence (5'-3')	Name	Sequence (5'-3')
mvaA	MvaA-Nde	AAGGCCTCATATGATTTCCCATACCCCGGT (SEQ ID NO:119)	MvaA-Bam	CGGGATCCTCATCGCTCCATCTCCATGT (SEQ ID NO:120)
idi	Idi-Nde	AAGGCCTCATATGACCGACAGCAAGGATCA (SEQ ID NO:121)	Idi-Bam	CGGGATCCTCATTGACGGATAAGCGAGG (SEQ ID NO:122)
hsc	Hcs-Nde	AAGGCCTCATATGAAAGTGCCTAAGATGA (SEQ ID NO:123)	Hcs-Bam	CGGGATCCTCAGGCCTGCCGGTCGACAT (SEQ ID NO:124)
mvk	Mvk-Nde[1]	AAGGCCTCATATGAGCACCGGCAGGCCTGAAGCA (SEQ ID NO:125)	Mvk-Bam[2]	CGGGATCCTCATCCCTGCCCCGGCAGCGGTT (SEQ ID NO:126)
pmk	Pmk-Nde	AAGGCCTCATATGGATCAGGTCATCCGCGC (SEQ ID NO:127)	Pmk-Bam	CGGGATCCTCAGTCATCGAAAACAAGTC (SEQ ID NO:128)
mvd	Mvd-Nde	AAGGCCTCATATGACTGATGCCGTCCGCGA (SEQ ID NO:129)	Mvd-Bam	CGGGATCCTCAACGCCCCTCGAACGGCG (SEQ ID NO:130)

[1]The second codon TCA was changed to AGC (silent mutation - both codons encode serine).

[2]The last codon GGC was changed to GGA (silent mutation - both codons encode glycine).

Table 19: Names of expression plasmids and construction intermediates.

Gene	PCR fragments in pCR®2.1-TOPO	After first mutagenesis	After 2nd mutagenesis	Genes in pDS	Genes in pDS-His
mvaA	TOPO-*mvaA*-BB	TOPO-*mvaA*-B	TOPO-*mvaA*	pDS-*mvaA*	pDS-His –*mvaA*
idi	TOPO-ORFX-B	TOPO-*idi*	n/a	pDS-*idi*	pDS-His -*idi*
hsc	TOPO-*hcs*-EN	TOPO-*hcs*-N	TOPO-*hcs*	pDS-*hcs*	pDS-His-*hcs*
mvk	TOPO-*mvk*	n/a	n/a	pDS-*mvk*	pDS-His -*mvk*
pmk	TOPO-*pmk*-B	TOPO-*pmk*	n/a	Nd	pDS-His -*pmk*
mvd	TOPO-*mvd*-B	TOPO-*mvd*	n/a	pDS-*mvd*	pDS-His -*mvd*

n/a: not applicable; nd: not done

Table 20: Oligonucleotides for site-directed mutagenesis.

Gene/ Site	Forward primer		Reverse primer	
	Name	Sequence (5'-3')	Name	Sequence (5'-3')
mvaA/ *Bam*HI-1	Mva-B1up	CCGGCATTCGGGCGGC ATCCAGGTCTCGCTG (SEQ ID NO:131)	Mva-B1down	CAGCGAGACCTGGATG CCGCCCGAATGCCGG (SEQ ID NO:132)
mvaA/ *Bam*HI-2	Mva-B2up	CGTGCAGGGCTGGATT CTGTCGGAATACCCG (SEQ ID NO:133)	Mva-B2down	CGGGTATTCCGACAGA ATCCAGCCCTGCACG (SEQ ID NO:134)
idi/ *Bam*HI	Idi-Bup2	GGGCTGCGCGCCGGCA TCCGGCATTTCGACG (SEQ ID NO:135)	Idi-Bdown2	CGTCGAAATGCCGGAT GCCGGCGCGCAGCCC (SEQ ID NO:136)
hcs/ *Eco*RI	Hcs-Eup	GGGTGCGACGGGCGAG TTCTTCGATGCGCGG (SEQ ID NO:137)	Hcs-Edown	CCGCGCATCGAAGAAC TCGCCCGTCGCACCC (SEQ ID NO:138)
hcs/ *Nde*I	Hcs-Nup-c	CACGCCCGTCACATAC GACGAATACGTTGCC (SEQ ID NO:139)	Hcs-Ndown-c	GGCAACGTATTCGTCG TATGTGACGGGCGTG (SEQ ID NO:140)
pmk/ *Bam*HI	Pmk-Bup	GAGGCTCGGGCTTGGC TCCTCGGCGGCGGTG (SEQ ID NO:141)	Pmk-Bdown	CACCGCCGCCGAGGAG CCAAGCCCGAGCCTC (SEQ ID NO:142)
mvd/ *Bam*HI	Mvd-Bup	CGGCACGCTGCTGGAC CCGGGCGACGCCTTC (SEQ ID NO:143)	Mvd-Bdown	GAAGGCGTCGCCCGGG TCCAGCAGCGTGCCG (SEQ ID NO:144)

E. coli strain M15 [Villarejo and Zabin, J. Bacteriol. 120:466-474 (1974)] carrying the *lacI* (lac repressor)-containing plasmid pREP4 (EMBL/GenBank accession number A25856) was transformed with the ligation mixtures and recombinant cells were selected for by

growth on LB-Agar plates supplemented with 100 mg/L ampicillin and 25 mg/L kanamycin. Positive clones containing the correct mevalonate operon gene insert were verified by PCR.

For expression of the inserted genes, each of the *E. coli* strains were grown overnight at 37°C in LB medium containing 25 mg/L kanamycin and 100 mg/L ampicillin. The next day, 25 ml of fresh medium was inoculated with 0.5 ml of the overnight cultures and the new cultures were grown at 37°C. When the OD_{600} of the cultures reached 0.4, expression of the cloned genes was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM, and the incubation of the cultures (with shaking) was continued for four hours, after which the cells were collected by centrifugation.

Crude extract preparation, HMG-CoA reductase assays, and IPP isomerase assays were performed as described in Example 1. Tables 21 and 22 show the HMG-CoA reductase and IPP isomerase activities, respectively, in the recombinant *E. coli* strains. Upon IPTG induction, strains M15/pDS-*mvaA* and M15/pDS-*idi* contained high levels of the HMG-CoA reductase and IPP isomerase activity, respectively. This illustrates the ability to over-express the mevalonate pathway genes (and overproduce their cognate gene products in an active form) from *Paracoccus* sp. strain R114 in *E. coli*.

Table 21. Induction of HMG-CoA reductase activity in *E. coli* strains over-expressing the cloned *mvaA* gene from *Paracoccus* sp. strain R114.

Strain/plasmid	IPTG Induction	HMG-CoA reductase activity (U/mg)
M15/pDS-*mvaA*	-	8.34
M15/pDS-*mvaA*	+	90.0
M15/pDS-His-*mvaA*	-	1.74
M15/pDS-His-*mvaA*	+	2.95
M15/pDS-*mvd*[a]	-	0.05

[a]M15/pDS-*mvd* was included as a negative control

Table 22. Induction of IPP isomerase activity in *E. coli* strains over-expressing the cloned *idi* gene from *Paracoccus* sp. strain R114.

Strain/plasmid	IPTG Induction	IPP isomerase activity (U/mg)
M15/pDS-*idi*	-	Not detected[b]
M15/pDS-*idi*	+	22.0
M15/pDS-His-*idi*	-	Not detected
M15/pDS-His-*idi*	+	Not detected
M15/pDS-*mvd*[a]	-	Not detected

[a]M15/pDS-*mvd* was included as a negative control

[b]<1 U/mg

The crude extracts used for the enzyme assays were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For strains *E. coli* M15/pDS-*mvaA* and *E. coli* M15/pDS-His-*mvaA*, the presence or absence of a highly expressed protein of the expected molecular mass (36.3 kD) correlated with the HMG-CoA reductase activity measured in the extracts (Table 21). The absence of the His-tagged protein could be explained by reduced expression at the level of transcription or translation by instability of the mRNA or the protein. The crude extracts of *E. coli* M15/pDS-*idi* and *E. coli* M15/pDS-His-*idi* both showed highly expressed proteins of the expected molecular masses of 37.3 kD and 39.0 kD, respectively. However, only the extract from *E. coli* M15/pDs-*idi* had increased IPP isomerase activity (Table 22), indicating that the histidine-tagged form of the enzyme was not functional under these conditions.

By SDS-PAGE analysis of crude extracts of *E. coli* strains over-expressing the other four genes of the *Paracoccus* sp. strain R114 mevalonate operon (*hcs*, *pmk*, *mvk*, and *mvd* , refer to Table 19) high expression of the native form of the enzyme was not detected upon IPTG induction, although some expression cannot be ruled out. On the other hand, high expression was observed with the His-tagged form of all four proteins.

Example 6: Improved Zeaxanthin Production in *Paracoccus* sp. strain R114 by Over-Expression of the *crtE* Gene

Construction of pBBR-K-Zea4, pBBR-K-Zea4-up and pBBR-K-Zea4-down and effects of these plasmids on zeaxanthin production in *Paracoccus* sp. strain R114. The carotenoid (*crt*) gene cluster of *Paracoccus* sp. strain R1534 was excised from plasmid pZea-4 [Pasamontes et al., Gene 185:35-41 (1997)] as an 8.3 kb *Bam*HI - *Eco*RI fragment. This fragment containing the *crt* gene cluster was ligated into the *Bam*HI and *Eco*RI-cut vector pBBR1MCS-2 (GenBank accession #U23751) resulting in pBBR-K-Zea4. Plasmid pBBR-

K-Zea4 was introduced into *Paracoccus* sp. strain R114 by conjugation to test for improved zeaxanthin production. The control strain R114 and two independent isolates of strain R114/pBBR-K-Zea4 were tested for zeaxanthin production in shake flask cultures (using medium 362F/2, see Example 11). The data in Table 23 show that both recombinant strains carrying plasmid pBBR-K-Zea4 produced significantly higher levels of zeaxanthin than R114, and had higher specific rates of production (mg zeaxanthin/OD_{660}). This suggested that one or more of the genes within the cloned insert in pBBR-K-Zea4 encoded an enzyme(s) that was limiting for zeaxanthin production in *Paracoccus* sp. strain R114.

Table 23. Zeaxanthin production by strains R114 and R114/pBBR-K-Zea4.

	24 Hours		48 Hours		72 Hours	
Strain	ZXN[a] (mg/l)	Spec. Form.[b]	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	54.5	2.2	81.7	4.1	78.1	4.5
R114/pBBR-K-Zea4 (clone 4)	41.0	3.0	100.7	5.2	97.6	6.2
R114/pBBR-K-Zea4 (clone 5)	41.1	3.1	110.5	5.7	102.1	6.5

[a]Zeaxanthin
[b]Specific Formation (mg ZXN/l/OD_{660})

To localize the positive effect, two plasmid derivatives were created that contained sub-cloned regions of the cloned insert present in pBBR-K-Zea4. The "upstream" region of the pBBR-K-Zea4 insert, comprising ORF 5 and the genes *atoB* and *crtE*, (Pasamontes et al., supra) is flanked by unique sites for the restriction enzymes *Xba*I and *Avr*II. Plasmid pBBR-K-Zea4-down was constructed by digesting pBBR-K-Zea4 with these two enzymes and deleting the "upstream" region. Analogously, plasmid pBBR-K-Zea4-up was constructed by deletion of the "downstream" region within the cloned insert in pBBR-K-Zea4, using the restriction enzymes *Eco*RV and *Stu*I. The two new plasmids were transferred to *Paracoccus* sp. strain R114 by conjugation. Zeaxanthin production was compared (shake flask cultures, same conditions as described above) in strains R114 (host control), R114/pBBR-K (empty vector control), R114/ pBBR-K-Zea4-down and R114/pBBR-K-Zea4-up (Table 24). The data clearly showed that the positive effect on zeaxanthin production was a result of the presence in multiple copies of the cloned segment containing ORF5, *atoB* and *crtE* , i.e., the insert present in plasmid pBBR-K-Zea4-up. A series of deletion plasmids was constructed from pBBR-K-Zea4-up. By introducing each of these plasmids into strain R114 and testing for zeaxanthin production, it was determined that it

was over-expression of the *crtE* gene that was providing the improved zeaxanthin production in strains R114/pBBR-K-Zea4 and pBBR-K-Zea4-up. This result is consistent with the activity of GGPP synthase (encoded by *crtE*) being limiting for zeaxanthin production in *Paracoccus* sp. strain R114. Using the methods described in Example 1, crude extract of strain R114/pBBR-K-Zea4-up was found to have 2.6-fold higher GGPP synthase activity than R114. To prove this directly, a new plasmid allowing over-expression of only the *crtE* gene was constructed as described in the following two sections.

Table 24. Zeaxanthin production by strains carrying deletion derivatives of plasmid pBBR-K-Zea4.

	24 Hours		48 Hours		72 Hours	
Strain	ZXN[a] (mg/l)	Spec. Form.[b]	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	35.0	1.2	75.7	4.1	73.9	4.4
R114/pBBR-K	32.0	1.5	59.3	3.1	63.3	3.9
R114/pBBR-K-Zea4-up	51.5	2.2	98.8	5.5	85.5	5.7
R114/pBBR-K-Zea4-down	41.6	1.8	63.4	3.3	66.4	3.9

[a]Zeaxanthin

[b]Specific Formation (mg ZXN/l/OD_{660})

Construction of the expression vectors pBBR-K-P*crtE* and pBBR-tK-P*crtE*. The vector pBBR1MCS-2 was cut with *Bst*XI and *Bsu*36I and the larger fragment was ligated with the annealed oligonucleotides MCS-2 up (5' TCAGAATTCGGTACCATATGAAGCTTGGATCCGGGG 3') (SEQ ID NO:145) and MCS-2 down (5' GGATCCAAGCTTCATATGGTACCGAATTC 3') (SEQ ID NO:146), resulting in vector pBBR-K-Nde. The 270 bp region upstream of the *crtE* gene in the carotenoid gene cluster from *Paracoccus* sp. strain R114, which contains the putative *crtE* promoter (P*crtE*) including the ribosome binding site and the *crtE* start codon (Pasamontes et al., supra) was amplified from *Paracoccus* sp. strain R114 DNA by PCR with primers crtE-up (5' GGAATTCGCTGCTGAACGCGATGGCG 3') (SEQ ID NO:147) and crtE-down (5' GGGGTACCATATGTGCCTTCGTTGCGTCAGTC 3') (SEQ ID NO:148). The PCR product was cut with *Eco*RI and *Nde*I and inserted into the *Eco*RI-*Nde*I cut backbone of pBBR-K-Nde, yielding plasmid pBBR-K-P*crtE*. An *Nde*I site, which contains the ATG start codon of *crtE*, was included in primer crtE-down. Hence, any introduced coding region with the start codon embedded in a *Nde*I site should be expressed using the ribosomal binding site of *crtE*. The plasmid pBBR-K-P*crtE* was cut with *Bam*HI and the annealed oligonucleotides pha-t-up

(5' GATCCGGCGTGTGCGCAATTTAATTGCGCACACGCCCCCTGCGTTTAAAC 3') (SEQ ID NO:149) and pha-t-down (5' GATCGTTTAAACGCAGGGGGCGTGTGCGCAATTAAATTGCGCACACGCCG 3') (SEQ ID NO:150) were inserted. The insertion was verified by sequencing, and the version of the plasmid having the oligos inserted in the orientation that reconstitutes the *Bam*HI site closer to the P*crtE* promoter was named pBBR-tK-P*crtE*. The inserted sequence carries the putative transcriptional terminator found between the *Paracoccus* sp. strain R114 *phaA* and *phaB* genes (see Example 10) and should, therefore, ensure proper termination of the transcripts initiated from the P*crtE* promoter.

<u>Construction of plasmid pBBR-K-P*crtE*-*crtE*-3</u>. To construct a multi-copy plasmid for increased expression of the *crtE* gene in the *Paracoccus* sp. strain R114 host, the *crtE* gene was amplified from plasmid p59-2 (Pasamontes et al., supra) by PCR using the primers crtE-Nde (5' AAGGCCTCATATGACGCCCAAGCAGCAATT 3') (SEQ ID NO:151) and crtE-Bam (5' CGGGATCCTAGGCGCTGCGGCGGATG 3') (SEQ ID NO:152). The amplified fragment was cloned in the pCR®2.1-TOPO vector, resulting in plasmid TOPO-*crtE*. The *Nde*I-*Bam*HI fragment from TOPO-*crtE* was subcloned in *Nde*I-*Bam*HI-digested plasmid pBBR-K-*PcrtE*, yielding pBBR-K-PcrtE-*crtE*. Finally, pBBR-K-P*crtE*-*crtE*-3 was constructed by replacing the smaller *Bgl*II fragment from pBBR-K-P*crtE*-*crtE* with the smaller *Bgl*II fragment from pBBR-K-Zea4-up. Plasmid pBBR-K-P*crtE*-*crtE*-3 was transferred to *Paracoccus* sp. strain R114 by electroporation. Using the methods described in Example 1, GGPP synthase activity in crude extracts was found to be 2.9-fold higher in strain R114/pBBR-K-P*crtE* –*crtE* –3 than in strain R114. This degree of elevated activity was similar to that observed in R114/pBBR-K-Zea4-up. Table 25 shows the zeaxanthin production by strain R114/pBBR-K-P*crtE* –*crtE* –3 was essentially identical to strain R114/pBBR-K-Zea4-up.

Table 25. Comparison of zeaxanthin production by strains R114/pBBR-K-P*crtE* –*crtE* –3 and R114/pBBR-K-Zea4-up.

	24 Hours		48 Hours		72 Hours	
Strain	ZXN[a] (mg/l)	Spec. Form.[b]	ZXN (mg/l)	Spec. Form.	ZXN (mg/l)	Spec. Form.
R114	49.0	1.6	83.9	3.3	97.8	4.3
R114/pBBR-K	42.6	1.8	73.7	3.8	88.8	4.9
R114/pBBR-K-P*crtE* –*crtE* –3	64.6	2.9	127.0	5.8	165.6	8.5
R114/pBBR-K-Zea4-up	64.7	2.9	118.0	5.9	158.0	10.1

[a]Zeaxanthin

[b]Specific Formation (mg ZXN/l/OD_{660})

Example 7: Expression of Individual Genes of the *Paracoccus* sp. strain R114 Mevalonate Operon in the Native Host, *Paracoccus* sp. strain R114

Expression of individual cloned genes of the *Paracoccus* sp. strain R114 mevalonate operon in the *Paracoccus* sp. strain R114 host. The mutagenized coding regions of the mevalonate operon genes in TOPO-plasmids (see Example 5) were excised with *Bam*HI and *Nde*I and ligated with the *Bam*HI-*Nde*I cleaved vector pBBR-tK-P*crtE* (see Example 6). The resulting plasmids pBBR-tK-P*crtE-mvaA*, pBBR-tK-P*crtE-idi*, pBBR-tK-P*crtE-hcs*, pBBR-tK-P*crtE-mvk*, pBBR-tK-P*crtE-pmk* and pBBR-tK-P*crtE-mvd* were introduced into *Paracoccus* sp. strain R114 by electroporation. Transformants were selected on agar medium containing 50 mg/l kanamycin and verified by PCR.

To illustrate that the plasmid-borne mevalonate pathway genes can be expressed in the native host *Paracoccus* sp. strain R114, HMG-CoA reductase activity was compared in crude extracts of strains R114/pBBR-K (control) and R114/pBBR-tK-P*crtE-mvaA* (methods used are set forth in Example 1). The specific activities of HMG-CoA reductase in strains R114/pBBR-K and R114/pBBR-tK-P*crtE-mvaA* were 2.37 U/mg and 6.0 U/mg, respectively. Thus the presence of the *mvaA* gene on a multicopy plasmid (and expressed from the P*crtE* promoter) resulted in a 2.5-fold increase in HMG-CoA reductase activity relative to the basal (i.e., chromosomally encoded) activity of R114 carrying the empty vector pBBR-K.

Example 8: Construction of "Mini-Operons" for Simultaneous Over-Expression the Cloned Genes of the Mevalonate Pathway with the *Paracoccus* sp. strain R114 *crtE* Gene

Plasmid constructions. As was shown in Example 6, introduction of plasmid pBBR-K-P*crtE*-*crtE*-3 into *Paracoccus* sp. strain R114 resulted in increased production of zeaxanthin, indicating that GGPP synthase activity was rate limiting for zeaxanthin biosynthesis in strain R114. Example 7 further showed that genes coding for the enzymes of the mevalonate pathway could be over-expressed in the native host *Paracoccus* sp. strain R114, and resulted in increased activity of the encoded enzyme. However, none of the recombinant strains of *Paracoccus* sp. strain R114 that carried plasmids containing each individual gene of the mevalonate operon showed increased zeaxanthin production compared to strain R114. It is possible that the benefit of over-expression of the genes of the mevalonate operon in *Paracoccus* sp. strain R114 could be masked by the downstream "bottleneck" in the zeaxanthin pathway (GGPP synthase). Creation of plasmids that allow simultaneous over-expression of each mevalonate pathway gene (or perhaps combinations of these genes) together with *crtE* could relieve all rate limitations in the overall zeaxanthin biosynthetic pathway, thereby improving zeaxanthin production. The next section describes the construction of "mini-operons" designed to allow co-over-expression of *crtE* and each of the genes coding for the five enzymes of the mevalonate pathway.

The *crtE*, *mvaA*, *idi* and *mvk* genes were excised from the respective TOPO-plasmids (described in Examples 5 and 6) with *Bam*HI and *Nde*I and ligated with *Bam*HI-*Nde*I-cleaved vector pOCV-1 (described in Example 12). The *crtE* gene does not have an adenine as the last nucleotide of the coding region, and in addition, has a TAG rather than a TGA stop codon and an unsuitable distance between the stop codon and the *Bam*HI site. Therefore, the end of *crtE* does not meet the requirements of the operon construction vectors (refer to Example 12) and *crtE* must be the last gene in any operon constructed with pOCV-1-*crtE*. To meet the requirement for an adenine as the first nucleotide of the second codon and the last nucleotide of the last codon, mutations had to be introduced in three genes of the mevalonate operon. The second codon of *pmk*, GAT, encoding Asp, was changed into AAT, encoding Asn. The last codon of *mvd* ends with a T and the last codons of *pmk* and *hcs* end with C. Changing these nucleotides to A results in silent mutations except for *pmk* where the last amino acid is changed from Asp to Glu. Oligonucleotides were designed to introduce the necessary changes by PCR. The sequences of the oligonucleotides and the templates used for those PCR reactions are shown in Table 26. All PCR products were cloned in the pCR®2.1-TOPO vector, resulting in plasmids TOPO-*mvd*^{OCV}, TOPO-*pmk*^{OCV} and TOPO-*hcs*^{OCV}. The inserts were excised

with *Nde*I and *Bam*HI and ligated with the *Nde*I-*Bam*HI cut backbone of pOCV-2 (see Example 12). The final cloning steps to assemble each of the "mini-operons" were analogous, and are illustrated by the representative scheme for construction of pBBR-K-P*crtE*-*mvaA*-*crtE*-3.

Table 26: Oligonucleotides and templates used for PCR in the construction of plasmids TOPO-*mvd*^{OCV}, TOPO-*pmk*^{OCV} and TOPO-*hcs*^{OCV}.

Gene	Forward primer		Reverse primer		Template
	Name	Sequence (5'-3')	Name	Sequence (5'-3')	
Hcs	Hcs-Nde	AAGGCCTCATATGAAAGTGCCTAAGATGA (SEQ ID NO:123)	Hcs-mut3	CCGGATCCTCATGCCTGCCGGTCGACATAG (SEQ ID NO:153)	pBBR-tK-P*crtE*-*hcs*
Pmk	Pmk-mut5	GAAGGCACATATGAATCAGGTCATCCGCGC (SEQ ID NO:154)	Pmk-mut3	GCCGGATCCTCATTCATCGAAAACAAGTCC (SEQ ID NO:155)	pBBR-tK-P*crtE*-*pmk*
Mvd	Mvd-Nde	AAGGCCTCATATGACTGATGCCGTCCGCGA (SEQ ID NO:129)	Mvd-mut3	ACGCCGGATCCTCATCGCCCCTCGAACGGC (SEQ ID NO:156)	pBBR-tK-P*crtE*-*mvd*

Example 9: Cloning and Sequencing of the *ispA* Gene Encoding FPP Synthase from *Paracoccus* sp. strain R114

Because FPP synthase lies in the central pathway for zeaxanthin biosynthesis in *Paracoccus* sp. strain R114, increasing the activity of this enzyme by increasing the dosage of the *ispA* gene has the potential to improve zeaxanthin production. For this reason, the *ispA* gene from *Paracoccus* sp. strain R114 was cloned and sequenced as follows. The amino acid sequences of six bacterial FPP synthases were obtained from public databases. These sequences have several highly conserved regions. Two such regions, and the oligonucleotides used for PCR, are shown in Table 27. PCR with oligonucleotides GTT-1 and GTT-2, using *Paracoccus* sp. strain R114 DNA as template, gave a product of the expected size. The PCR product was cloned in the vector pCR®2.1-TOPO and sequenced. The cloned fragment was used as a probe for a Southern analysis of *Paracoccus* sp. strain R114 DNA and was found to hybridize to a *Bam*HI-*Nco*I fragment of about 1.9 kb. *Paracoccus* sp. strain R114 DNA was cut with *Bam*HI and *Nco*I and the fragments were separated by agarose gel electrophoresis. The region between 1.5 and 2.1 kb was isolated and cloned in the *Bam*HI and *Nco*I sites of a cloning vector. This partial library was then screened using the *ispA*-PCR fragment as a probe, and two positive clones were isolated. Sequencing confirmed that the plasmids of both clones contained the *ispA* gene. Upstream of *ispA* (SEQ ID NO:159) is the gene for the small subunit of exonuclease VII, XseB (SEQ ID NO:158),

and downstream is the *dxs* gene (SEQ ID NO:160) encoding the 1-deoxyxylulose-5-phosphate synthase. This is the same gene arrangement as found in *E. coli*. The sequence of the *Nco*I-*Bam*HI fragment is illustrated in SEQ ID NO:157, the amino acid sequences of XseB, IspA and Dxs are illustrated in SEQ ID NO:158, SEQ ID NO:159, and SEQ ID NO:160, respectively. The start codon of *ispA* may be GTG or ATG resulting in two or one methionine residues, respectively, at the amino-terminus of the native IspA.

Using the same general cloning strategy described in Examples 5-7, a new plasmid, pBBR-tK-P*crtE*-*ispA*-2 was constructed to allow for over-expression of the *ispA* gene in the native host *Paracoccus* sp. strain R114. The plasmid was introduced into strain R114 by electroporation, and transformants were confirmed by PCR. Three representative transformants and a control strain (R114/pBBR-K) were grown in 362F/2 medium (Example 11), crude extracts were prepared and assayed for activity of the *ispA* gene product, FPP synthase according to the methods described in Example 1. The basal (chromosomally-encoded) FPP synthase specific activity in R114/pBBR-K was 62.6 U/mg. The FPP synthase activity in the three transformants was 108.3 U/mg (73% increase), 98.5 U/mg (57% increase) and 83.8 U/mg (34% increase), demonstrating the over-expression of the *ispA* gene and over-production of its product, FPP synthase, in an active form in *Paracoccus* sp. strain R114.

Table 27: Oligonucleotides designed from two conserved bacterial IspA peptides.

<u>Peptide 1</u>	
Bradyrhizobium japonicum	Val His Asp Asp Leu Pro (SEQ ID NO:161)
Rhizobium sp. strain NGR234	Val His Asp Asp Leu Pro (SEQ ID NO:162)
Bacillus stearothermophilus	Ile His Asp Asp Leu Pro (SEQ ID NO:163)
Bacillus subtilis	Ile His Asp Asp Leu Pro (SEQ ID NO:164)
Escherichia coli	Ile His Asp Asp Leu Pro (SEQ ID NO:165)
Haemophilus influenzae	Ile His Asp Asp Leu Pro (SEQ ID NO:166)
Oligonucleotide GTT-1 (5'-3')	tc cay gay gay ctg cc (SEQ ID NO:167)
Peptide 2	
Bradyrhizobium japonicum	Asp Asp Ile Leu Asp (SEQ ID NO:168)
Rhizobium sp. strain NGR234	Asp Asp Ile Leu Asp (SEQ ID NO:169)
Bacillus stearothermophilus	Asp Asp Ile Leu Asp (SEQ ID NO:170)
Bacillus subtilis	Asp Asp Ile Leu Asp (SEQ ID NO:171)
Escherichia coli	Asp Asp Ile Leu Asp (SEQ ID NO:172)
Haemophilus influenzae	Asp Asp Ile Leu Asp (SEQ ID NO:173)
Reverse complement of Oligonucleotide GTT-2 (5'-3')	gay gay atc ctg gay (SEQ ID NO:174)

Y = C or T

Example 10: Cloning and Sequencing of the Genes Coding for Acetyl-CoA Acetyltransferase from *Paracoccus* sp. strain R114

The first committed step in IPP biosynthesis is the condensation of acetyl-CoA and acetoacetyl-CoA to hydroxymethylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase. The substrate acetoacetyl-CoA is formed by the enzyme acetyl-CoA acetyltransferase (also known as acetoacetyl-CoA thiolase or β-ketothiolase) by condensation of two molecules of acetyl-CoA. Because this reaction links central metabolism (at acetyl-CoA) to isoprenoid biosynthesis via the mevalonate pathway, increasing the activity of acetyl-CoA acetyltransferase by gene amplification has the potential to increase carbon flow to carotenoids and other isoprenoids *in vivo*. In *Paracoccus* sp. strain R114, there are at least two genes, *atoB* and *phaA*, that encode acetyl-CoA acetyltransferases. The end of the *atoB* gene is 165 nucleotides upstream of the start of *crtE* in *Paracoccus* sp. strains R1534 (US 6,087,152) and R114 (this work). The nucleotide sequence of the *atoB* gene and the corresponding amino acid sequence of the encoded acetyl-CoA acetyltransferase from *Paracoccus* sp. strain R1534 are illustrated in SEQ ID NO:175 and SEQ ID NO:176, respectively.

Using the same general strategy as described in Example 5, the *atoB* gene was cloned in plasmids pDS and pDS-His. The new plasmids, pDS-*atoB* and pDS-His-*atoB* were introduced into *E. coli* strain M15. The resulting strains M15/pDS-*atoB* and M15/pDS-His-*atoB* were grown with and without IPTG induction (as described in Example 5), and crude extracts were prepared for acetyl-CoA acetyltransferase assays (methods used were described in Example 1) and SDS-PAGE analysis. The acetyl-CoA acetyltransferase specific activities in extracts of M15/pDS-*atoB* and M15/pDS-His-*atoB* (with IPTG induction) were 0.2 U/mg and 13.52 U/mg, respectively. The basal activity measured in *E. coli* without the plasmids was 0.006 U/mg. Upon IPTG induction the *atoB* gene product, acetyl-CoA acetyltransferase, is overproduced in *E. coli* M15. Both the native (M15/pDS-*atoB*) and His-tagged (M15/pDS/his-*atoB*) forms were overproduced. The degree of overproduction was much higher in M15/pDS-His-*atoB*, consistent with the measured acetyl-CoA acetyltransferase activity in the (induced) extracts of the two strains.

Acetoacetyl-CoA is also the substrate for the first committed step in polyhydroxyalkanoate (PHA) biosynthesis. In many bacteria the genes involved in PHA biosynthesis are grouped in operons [Madison and Huisman, Microbiol. Mol. Biol. Rev., 63:21-53 (1999)]. In *Paracoccus denitrificans* the *phaA* and *phaB* genes, encoding the acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, respectively, are clustered in

an operon [Yabutani et al., FEMS Microbiol. Lett. 133:85-90 (1995)] whereas *phaC*, the gene encoding the last enzyme in the pathway, poly(3-hydroxyalkanoate) synthase, is not part of this operon [Ueda et al., J. Bacteriol. 178:774-779 (1995)]. PCR fragments containing parts of *phaA* from *Paracoccus* sp. strain R1534 and *phaC* from *Paracoccus* sp. strain R114 were obtained using primers based on the *P. denitrificans phaA* and *phaC* gene sequences. The PCR fragments were then used as probes to screen a *Paracoccus* sp. strain R114 λ-library (see Example 4). Several λ-clones hybridizing with the *phaA* or the *phaC* probes were isolated, and the presence of the *phaA* or *phaC* genes in the inserts was verified by sequence analysis. Three *phaA* λ-clones were further analyzed by subcloning and sequencing, whereby the *phaB* was found downstream of *phaA*. Therefore, as is the case in *P. denitrificans*, the *phaA* and *phaB* genes are clustered whereas the *phaC* gene is located elsewhere in the genome. The nucleotide sequence of the *phaAB* cluster from *Paracoccus* sp. strain R114 and the deduced amino acid sequences of the acetyl-CoA acetyltransferase (PhaA) are illustrated in SEQ ID NO:177, and SEQ ID NOs:178 and 179, respectively. The clustering of genes involved in PHA biosynthesis in operons suggests that at least *phaA* and *phaB* are expressed together when the cell produces poly(3-hydroxyalkanoates). On the other hand, a putative transcriptional stop signal is found between the *phaA* and *phaB* genes from *Paracoccus* sp. strain R114 which is absent in the *P. denitrificans phaAB* operon (Yabutani et al., supra). Thus, the expression of the two genes might not be coupled in *Paracoccus* sp. strain R114.

Using the same general strategy as described in Example 5, the *phaA* gene was cloned in plasmid pDS-His. The new plasmid, pDS-His-*phaA*, was introduced into *E. coli* strain M15. The resulting strain M15/pDS-His-*phaA* was grown with and without IPTG induction (as described in Example 5) and crude extracts were prepared for SDS-PAGE analysis. The cloned His-tagged *Paracoccus* sp. strain R114 PhaA (acetyl-CoA acetyltransferase) is overproduced upon IPTG induction in the *E. coli* M15 host.

The potential benefit of amplifying the *atoB* or *phaA* genes, encoding acetyl-Co acetyltransferase, on zeaxanthin production is mentioned above. In addition, it may be beneficial for zeaxanthin production to decrease or eliminate the activity of actoacetyl-CoA reductase (the *phaB* gene product) to avoid diversion of some of the acetoacetyl-CoA formed *in vivo* to the PHA pathway. Mutants of *Paracoccus* sp. strain R114 lacking activity of *phaB* could be obtained by gene replacement techniques (specifically replacing the wild-type *phaB* gene in the chromosome with an inactive form of the gene) or by classical mutagenesis and screening.

Example 11: Model for the Industrial Production of Lycopene Using Mutants Derived from *Paracoccus* sp. strain R114

Lycopene is a red carotenoid that is an intermediate in the biosynthesis of zeaxanthin in the new *Paracoccus* species represented by strain R-1512 and its mutant derivatives R1534 and R114. As lycopene itself has significant commercial potential, it was of interest to test the potential of the new *Paracoccus* species to produce lycopene by industrial fermentation. To obtain mutants blocked in zeaxanthin biosynthesis that accumulated lycopene, *Paracoccus* sp. strain R114 was subjected to mutagenesis with ultraviolet (UV) light followed by screening for red colonies. The UV mutagenesis was performed as follows. An overnight culture of strain R114 was grown in ME medium (see Example 2). The overnight culture was subcultured into fresh ME medium (initial $OD_{610} = 0.1$) and incubated at 28°C for 3 hours. Aliquots of this culture were centrifuged and the pellet washed with 20mM potassium phosphate buffer (pH 7.2). After a second centrifugation, the pellet was resuspended to a final OD_{610} of 0.1. Ten milliliter aliquots of the cell suspension were placed in a sterile 100-ml glass beaker. The thin layer of cell suspension was irradiated with UV light at a flux of 1450μW/cm^2 for a pre-determined optimal length of time. The cell suspension was mixed during the irradiation by means of a paper clip in the beaker and a magnetic stirrer. The mutagenized cell suspensions (and the unmutagenized controls) were plated on 362/F2 agar medium (Table 28). Triplicate viable plate counts (in dim room light) were done on suspensions before and after mutagenesis. Plates were incubated for 4-5 days at 28°C, and the colonies were scored. Several red colonies (putative lycopene producers) were identified and purified by re-streaking. One mutant, designated UV7-1, was further evaluated for lycopene production.

Table 29 shows the zeaxanthin production and lycopene production by the control strain R114 and its mutant derivative UV7-1. Strain R114 produced only zeaxanthin. Mutant UV7-1 produced mostly lycopene, but also produced a residual amount of zeaxanthin, suggesting that the mutational block in UV7-1 (presumably in the *crtY* gene) is not complete. These results show that it is possible to derive lycopene producing strains from *Paracoccus* sp. strain R114.

Table 28. Recipe and preparation for medium 362F/2

Component	Amount
Glucose monohydrate	33 g
Yeast extract	10 g
Tryptone	10 g
NaCl	5 g
$MgSO_4 \cdot 7H_2O$	2.5 g
Agar (for solid medium)	20 g
Distilled water	To 932 ml
-adjust pH to 7.4	
-sterilize by filtration (liquid medium) or autoclaving (solid medium)	
-Add 2.5 ml each of microelements solution, NKP solution and CaFe solution	
Microelements solution	**Amount per liter distilled water**
$(NH_4)_2Fe(SO4)_2 \cdot 6H_2O$	80 g
$ZnSO_4 \cdot 7H_2O$	6 g
$MnSO_4 \cdot H_2O$	2 g
$NiSO_4 \cdot 6H_2O$	0.2 g
EDTA	6 g
-sterilize by filtration	
NKP solution	**Amount per liter distilled water**
K_2HPO_4	250 g
$(NH_4)_2HPO_4$	300 g
-sterilize by filtration	
CaFe solution	**Amount per liter distilled water**
$CaCl_2 \cdot 2H_2O$	75 g
$FeCl_3 \cdot 6H_2O$	5 g
Concentrated HCl	3.75 ml
-sterilize by filtration	

Table 29. Zeaxanthin and lycopene production by *Paracoccus* sp. strain R114 and its red mutant derivative UV7-1.

	Zeaxanthin (mg/l)	Lycopene (mg/l)
24 hours		
R114	36.65	0
UV7-1	3.85	20.85
48 hours		
R114	72.95	0
UV7-1	5.75	85.95
72 hours		
R114	83.9	0
UV7-1	5.85	124.55

Example 12: Model for the Industrial Production of Astaxanthin by Fermentation Using Strains Derived from *Paracoccus* sp. strain R114

Astaxanthin is a commercially important carotenoid used primarily in the aquaculture industry. EP 872,554 showed that astaxanthin production could be achieved in *E. coli* by introducing plasmids containing combinations of the cloned carotenoid (*crt*) genes from *Paracoccus* sp. strain R1534 and *Paracoccus carotinifaciens* E-396^T. Together, the cloned *crt* genes (*crtEBIYZ*) and *crtW* (β-carotene β-4 oxygenase) encoded a total biosynthetic pathway from FPP through zeaxanthin to astaxanthin. The sequences of the *P. carotinifaciens* E-396 *crtW*, *Paracoccus* sp. R1534 *crtZ*, and *Paracoccus* sp. R1534 *crtE* genes and encoded polypeptides are set forth in (SEQ ID NOs:180 and 181 (*crtW*); 182 and 184 (*crtZ*); and 184 and 185 (*crtE*)) However, it was not shown that astaxanthin production could be achieved in the *Paracoccus* sp. strain R114 host family. To demonstrate the utility of recombinant strains derived from strain R114 for astaxanthin production, the cloned *crtW* gene (SEQ ID NO:180) was introduced into strain R114 as follows.

Table 30. PCR primers used for the work described in Example 12.

Primer name	Sequence
CrtW-Nde	5' AAGGCCTCATATGAGCGCACATGCCCTGCC 3' (SEQ ID NO:186)
CrtW-Bam	5' CGGGATCCTCATGCGGTGTCCCCCTTGG 3' (SEQ ID NO:187)
CrtZ-Nde	5' AAGGCCTCATATGAGCACTTGGGCCGCAAT 3' (SEQ ID NO:188)
CrtZ-Bam	5' AGGATCCTCATGTATTGCGATCCGCCCCTT 3' (SEQ ID NO:189)

The *crtW* gene was amplified by PCR from the cloned *crt* cluster of *Paracoccus carotinifaciens* strain E-396^T (Tsubokura et al., supra; EP 872,554) using the primers crtW-Nde and crtW-Bam (Table 30). The primers were designed such that the ATG start codon constitutes the second half of an *Nde*I site (cleavage recognition site CATATG), and a *Bam*HI site (GGATCC) was introduced immediately after the stop codon. The PCR product was cloned in the pCR®2.1-TOPO vector, resulting in plasmid TOPO-*crtW*. The *crtW* gene was excised with *Nde*I and *Bam*HI and subcloned in the *Nde*I-*Bam*HI cut vector pBBR-K-P*crtE* (described in Example 6) to create plasmid pBBR-K-P*crtE-crtW*.

Plasmid pBBR-K-P*crtE-crtW* was transferred to *Paracoccus* sp. strain R114 using a standard bacterial conjugation procedure {*E. coli* strain S17 [Priefer et al., J. Bacteriol. 163:324-330 (1985)] was the donor organism}. Transconjugants were selected on medium 362F/2 agar (Table 28) containing 50 mg/l kanamycin and purified by restreaking on the same medium. The presence of plasmid pBBR-K-P*crtE-crtW* in the strain was confirmed by PCR. Carotenoid production by strains R114 (host control), R114/pBBR-K (empty vector control) and R114/ pBBR-K-P*crtE-crtW* was measured in shake flask cultures as described in Examples 1 and 2, except that liquid 362F/2 medium was used instead of ME medium. These results are shown in Table 31. The control strains R114 and R114/pBBR-K produced only zeaxanthin. In strain R114/ pBBR-K-P*crtE-crtW*, the zeaxanthin was completely consumed by the plasmid-encoded β-carotene β-4 oxygenase. However, although astaxanthin was produced, two other ketocarotenoids, adonixanthin and canthaxanthin, accumulated at higher levels. This indicated an imbalance *in vivo* of the β-carotene hydroxylase (encoded by the chromosomal *crtZ* gene in strain R114) and the cloned β carotene β-4 oxygenase (CrtW).

To test this hypothesis, two new plasmids were created that contained the *crtZ* and *crtW* genes together in mini-operons. The order of the genes was made different in the two constructs (i.e., *crtZ-crtW* and *crtW-crtZ*) to try and create different ratios of expression of the *crtZ* and *crtW* genes. The construction of the new plasmids required the assembly of a special set of cloning vectors as follows. A series of operon construction vectors (based on the vector pCR®2.1-TOPO) was designed to facilitate the assembly of genes (in this case, *crtZ* and *crtW*) into operons. The genes of interest must have an ATG start codon, embedded in an *Nde*I site (CATATG), and a TGA stop codon immediately followed by a *Bam*HI site.

Table 31. Astaxanthin production in *Paracoccus* sp. strain R114 containing plasmids expressing the *crtW* gene alone and in combination with the *crtZ* gene.

	24 hours					
Strain	ZXN	ADN	CXN	AXN	Total	Sp. Form.[a]
R114	46.5	0	0	0	46.5	2.1
R114/pBBR-K	38.8	0	0	0	41.4	2.2
R114/pBBR-K-P*crtE* -*crtW*	0	13.0	21.8	2.3	37.5	2.1
R114/pBBR-K-P*crtE* -*crtWZ*	0	14.9	29.5	1.3	45.6	2.1
R114/pBBR-K-P*crtE* -*crtZW*	0	18.0	20.4	7.3	45.65	2.1
	48 hours					
R114	72.6	0	0	0	74.4	2.8
R114/pBBR-K	70.1	0	0	0	70.1	3.1
R114/pBBR-K-P*crtE* -*crtW*	0	26.7	22.0	26.9	75.5	3.9
R114/pBBR-K-P*crtE* -*crtWZ*	0	30.9	27.2	34.8	92.9	4.0
R114/pBBR-K-P*crtE* -*crtZW*	0	15.7	11.2	58.3	85.1	4.1
	72 hours					
R114	82.5	0	0	0	82.5	5.3
R114/pBBR-K	82.9	0	0	0	82.9	5.1
R114/pBBR-K-P*crtE* -*crtW*	0	19.7	17.0	46.8	83.5	5.2
R114/pBBR-K-P*crtE* -*crtWZ*	0	28.7	26.4	43.8	98.8	6.1
R114/pBBR-K-P*crtE* -*crtZW*	0	18.3	14.4	66.3	98.9	5.9

[a]ZXN, zeaxanthin; AND, adonixanthin; CXN, canthaxanthin; AXN, astaxanthin.
[b]Specific Formation, expressed as mg/l total carotenoid/OD_{660}.

Furthermore, the first nucleotide after the start codon and the last nucleotide before the stop codon must be adenine and the gene must lack sites for at least one of the enzymes *Bsg*I, *Bse*MII, *Bse*RI and *Gsu*I. Four operon construction vectors were constructed, differing in the arrangements of their polylinker sequences (SEQ ID NOs: 190-197). The cleavage sites of the first two enzymes are within the NdeI site. The cleavage sites of the last two enzymes are before the BamHI site. The BseRI site in pOCV-1 and pOCV-4 is not unique and cannot be used for operon construction.

The genes to be assembled in operons are first inserted individually between the *Nde*I and the *Bam*HI sites of the appropriate operon construction vectors. The resulting plasmid with the upstream gene of the envisioned operon is then cut with one of the two enzymes

at the end of the polylinker and with an enzyme, which has a unique site within the vector backbone. The plasmid containing the downstream gene of the envisioned operon is cut with one of the first two enzymes of the polylinker and with the same enzyme (with a unique site in the vector backbone) used for the first plasmid (containing the desired upstream gene). The fragments carrying the genes are isolated and ligated, resulting in a pOCV plasmid with both genes between the *Nde*I and the *Bam*HI sites. More genes can be added in an analogous fashion. The assembled genes overlap such that the first two nucleotides, TG, of the TGA stop codon of the upstream gene coincide the last two nucleotides of the ATG start codon of the downstream gene. The same overlap is found between all genes in the carotenoid (*crt)* operon (*crtZYIB*) in *Paracoccus* sp. strain R1534 (Pasamontes et al., supra).

The pOCV backbone is derived from pCR®2.1-TOPO. The *Bse*MII site in the region necessary for replication, upstream of the ColE1 origin, was eliminated by site directed mutagenesis changing the site from CTCAG into CACAG. The remaining three *Bse*MII sites and one *Gsu*I site were eliminated by removing a 0.8 kb *Dde*I-*Asp*700 fragment. The remaining vector was blunt-end ligated after fill-in of the *Dde*I recessed end. The polylinkers were inserted between the *Bam*HI and *Xba*I sites by means of annealed oligonucleotides with the appropriate 5' overhangs.

Plasmid pBBR-K-P*crtE-crtZW*, was constructed using the operon construction vector pOCV-2 as follows. The *crtZ* gene was amplified by PCR from *Paracoccus* sp. strain R114 using the primers crtZ-Nde and crtZ-Bam (Table 30). The primers were designed such that the ATG start codon constitutes the second half of a *Nde*I site (cleavage recognition site CATATG) and a *Bam*HI site (GGATCC) was introduced immediately after the stop codon. The PCR product was cloned in the pCR®2.1-TOPO vector, resulting in plasmid TOPO-*crtZ*. To assemble the two genes in a mini-operon, both genes, *crtZ* and *crtW* were excised with *Nde*I and *Bam*HI from the plasmids TOPO-*crtZ* and TOPO-*crtW* and subcloned in the *Nde*I-*Bam*HI cut vector pOCV-2, creating plasmids pOCV-2-*crtZ* and pOCV-2-*crtW*. Plasmid pOCV-2-*crtZ* was cut with *Bse*MII and *Pst*I (there is a unique *Pst*I site in the kanamycin resistance gene) and the 2.4 kb fragment (containing *crtZ*) was ligated with the *crtW*-containing 1876 bp *Bse*RI-*Pst*I fragment from pOCV-2-*crtW*. The resulting plasmid, pOCV-2-*crtZW*, was cut with *Nde*I and *Bam*HI and the *crtZW* fragment was ligated with the *Nde*I-*Bam*HI backbone of pBBR-K-P*crtE* to yield pBBR-K-P*crtE-crtZW*. Plasmid pBBR-K-P*crtE-crtWZ*, was constructed in an analogous fashion.

The data in Table 31 show that the ratio of adonixanthin, canthaxanthin and astaxanthin did not change appreciably in strain R114/ pBBR-K-P*crtE-crtWZ* compared to strain

pBBR-K-P*crtE-crtW*. However, in strain pBBR-K-P*crtE-crtZW*, the production of the ketocarotenoids was shifted in favor of astaxanthin. This result indicates that the level of expression is dependent on the position of the gene within the mini-operon, and suggests that increasing the *in vivo* level of β-carotene hydroxylase activity creates a balance between the activities of this enzyme and β-carotene β-4 oxygenase that is more favorable for full conversion of zeaxanthin to astaxanthin.

The results described in this Example also show that it is possible, through appropriate genetic engineering, to produce not only astaxanthin, but also other ketocarotenoids of commercial interest in *Paracoccus* sp. strain R114 or its relatives. For example, expression of a gene coding for β-carotene β-4 oxygenase in a *crtZ* mutant of strain R114 (lacking β-carotene hydroxylase activity) would provide for production of exclusively ketocarotenoids, *e.g.*, echinenone or canthaxanthin, without co-production of hydroxylated carotenoids. Taken together, the results presented in this Example and Example 11 show the broad utility of *Paracoccus* sp. strain R114 and its relatives to produce industrially important carotenoids.

Example 13: Accumulation of mevalonate in cultures of *Paracoccus* sp. strain R114 overexpressing genes of the mevalonate pathway

Overexpression of the genes of the mevalonate pathway in *Paracoccus* sp. strain R114 leads to increased carbon flow to through the mevalonate pathway. The construction of plasmid pBBR-K-mev-op16-2 was described in Example 5. Plasmid pBBR-K-mev-op-up-4 was constructed as follows. A DNA fragment containing containing most of the *mvaA* gene and the entire *idi* and *hcs* genes was obtained on a 3.1 kb *Sma*I-*Sal*I fragment following partial digestion of a λ-clone containing the *Paracoccus* sp. strain R114 mevalonate operon (see Example 4). This fragment was subcloned in pUC19, yielding the plasmid pUC19mev-op-up'. To facilitate subcloning, the *Kpn*I-*Hin*dIII fragment of pUC19mev-op-up' containing the mevalonate genes was recloned in the vector pBluescriptKS$^+$, resulting in plasmid pBluKSp-mev-op-up'. A 1.7 kb *Sal*I fragment from pUC19mev-op-up' was then cloned in the *Sal*I site of plasmid 2ES2-1, which is a pUC19-derived plasmid containing the cloned *Sal*I-*Eco*RI fragment M from *Paracoccus* sp. strain R114 (refer to Example 4). This resulted in plasmid pUC19mev-op-up-2. Plasmid pUCmev-op-up-3 was then obtained by combining the *Bbs*I-*Bsa*I fragment from pUC19mev-op-up-2 carrying the beginning of the mevalonate operon with the *Bbs*I-*Bsa*I fragment from pBluKSp-mev-op-up' containing *idi* and *hcs*. Separately, a unique *Mlu*I site was introduced between the *Nsi*I and *Kpn*I sites of the vector pBBR1MCS-2 (refer to Example 5) by inserting an annealed primer containing an *Mlu*I restriction site. The resulting new

cloning vector pBBR-K-Mlu was cut with *Mlu*I and *Kpn*I and the *Mlu*I-*Kpn*I fragment from pUCmev-op-up-3, containing the first three genes of the mevalonate operon, was inserted, yielding plasmid pBBR-K-mev-op-up-3. Plasmid pBBR-K-mev-op-up-4 was then constructed by insertion of the *Sma*I fragment from plasmid 16SB3, which contains most of the *mvk* gene and the 5' end of *pmk* (plasmid 16SB3 is a pUC19-derived plasmid containing the *Paracoccus* sp. strain R114 *Sal*I-*Bam*HI fragment A; refer to Example 4). The insert of plasmid pBBR-K-mev-op-up-4 contains the putative mevalonate operon promoter region, the first four genes of the mevalonate operon and the 5' end of *pmk* .

Plasmids pBBR-K-mev-op16-2 and pBBR-K-mev-op-up-4 were each introduced into *Paracoccus* sp. strain R114 by electroporation. Production of zeaxanthin and mevalonate by the new strains were compared to the control strain R114. The strains were grown in baffled shake flasks in liquid medium 362F/2 (see Example 11) for 72 hours. For strains R114/pBBR-K-mev-op16-2 and R114/pBBR-K-mev-op-up-4, kanamycin (50 mg/l) was also added to the cultures. The cultivation temperature was 28°C and shaking was at 200 rpm. Zeaxanthin was measured by the method set forth in Example 1, while mevalonate in the culture supernatants was measured as follows: A 0.6 ml sample of the culture was centrifuged for 4 minutes at 13,000 x g. Four hundred microliters of the supernatant were added to 400 microliters of methanol and mixed by vortexing for 1 min. The mixture was centrifuged again for 4 minutes at 13,000 x g. The resulting supernatant was then analyzed directly by gas chromatography (GC) using the method of Lindemann et al. [J. Pharm. Biomed. Anal. 9:311-316 (1991)] with minor modification as follows. The GC was a Hewlett-Packard 6890plus instrument (Hewlett-Packard, Avondale, PA, USA) equipped with a cool-on-column injector and a flame ionization detector. One microliter of sample prepared as described above was injected onto a fused silica capillary column (15m length x 0.32mm ID) coated with a 0.52 micron film of crosslinked modified polyethylene glycol (HP-FFAP, Agilent Technologies, USA). Helium was used as the carrier gas at an inlet pressure of 0.6 bar. The temperature of the programmable injector was ramped from 82°C to 250°C at a rate of 30°C/minute. The column temperature profile was 80°C for 0.5 minutes, followed by a linear temperature gradient at 15°C/min to 250°C and finally held at 250°C for 5 minutes. The detector temperature was maintained at 320°C.

In the first experiment, zeaxanthin and mevalonate production were measured in strains R114 and R114/pBBR-K-mev-op16-2 (Table 32). Both strains produced similar amounts of zeaxanthin, but strain R114/pBBR-K-mev-op16-2 produced a four-fold higher level of mevalonate. These results show that overexpression of the genes of the mevalonate pathway in *Paracoccus* sp. strain R114 results in increased carbon flow through the mevalonate

pathway. The accumulation of mevalonate was expected because strain R114/pBBR-K-mev-op16-2 does not have an overexpressed *crtE* gene, and the *crtE* gene product (GGPP synthase) is known to be a limiting step in zeaxanthin production in *Paracoccus* sp. strain R114 (see Examples 6 and 8). Cells having a limiting amount of GGPP synthase, upon overproduction of the enzymes of the mevalonate pathway, would be expected to accumulate FPP, and it is well known that FPP is a potent inhibitor of mevalonate kinase [Dorsey and Porter, J. Biol. Chem. 243:4667-4670 (1968); Gray and Kekwick, BBA 279:290-296 (1972); Hinson et al. J. Lipids Res. 38:2216-2223 (1997)]. Therefore, accumulation of FPP resulting from overexpression of the genes of the mevalonate pathway would cause inhibition of mevalonate kinase, which in turn is manifested as mevalonate accumulation in the culture.

Table 32. Zeaxanthin and mevalonate production in strains R114 and R114/pBBR-K-mev-op16-2.

Strain/plasmid	Mevalonate (mg/l)	Zeaxanthin(mg/l)
R114	50.5	70.0
R114/pBBR-K-mev-op16-2	208.2	65.2

In a second experiment, zeaxanthin and mevalonate production were measured in strain R114 and two independent isolates of R114/pBBR-K-mev-op-up-4 (Table 33). These results again show that overexpression of the genes of the mevalonate pathway increased carbon flow through the mevalonate pathway.

Table 33. Zeaxanthin and mevalonate production in strains R114 and R114/pBBR-K-mev-op-up-4.

Strain/plasmid	Mevalonate (mg/l)	Zeaxanthin(mg/l)
R114	45	67.5
R114/pBBR-K-mev-op-up-4 (Isolate 1)	133.2	53.7
R114/pBBR-K-mev-op-up-4 (Isolate 2)	163.7	47.6

The following biological material was deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, VA 20110-2201, USA, and were assigned the following accession numbers:

Strain	Accession No.	Date of Deposit
Paracoccus sp. R114	PTA-3335	April 24, 2001
Paracoccus sp. R1534	PTA-3336	April 24, 2001
Paracoccus sp. R-1506	PTA-3431	June 5, 2001

All patents, patent applications, and publications cited above are incorporated herein by reference in their entirety as if recited in full herein.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

<u>What is Claimed Is</u>:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
(a) an amino acid sequence shown as residues 1 to 340 of SEQ ID NO:43;
(b) an amino acid sequence shown as residues 1 to 349 of SEQ ID NO:45;
(c) an amino acid sequence shown as residues 1 to 388 of SEQ ID NO:47;
(d) an amino acid sequence shown as residues 1 to 378 of SEQ ID NO:49;
(e) an amino acid sequence shown as residues 1 to 305 of SEQ ID NO:51;
(f) an amino acid sequence shown as residues 1 to 332 of SEQ ID NO:53;
(g) a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, wherein said fragment has at least 30 contiguous amino acid residues;
(h) an amino acid sequence of a fragment of a polypeptide selected from the group consisting of SEQ ID NOs: 43, 45, 47, 49, 51, and 53, the fragment having the activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase;
(i) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides of SEQ ID NO:42 or a complement of SEQ ID NO:42, wherein the polypeptide has the activity of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; and
(j) a conservatively modified variant of SEQ ID NO:43, 45, 47, 49, 51 or 53.

2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
(a) an amino acid sequence shown as residues 1 to 287 of SEQ ID NO:159;
(b) at least 30 contiguous amino acid residues of SEQ ID NO:159;
(c) an amino acid sequence of a fragment of SEQ ID NO: 159, the fragment having the activity of farnesyl diphosphate synthase (FPP synthase);
(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 295-1158 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of FPP synthase; and
(e) a conservatively modified variant of SEQ ID NO:159.

3. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 142 of SEQ ID NO:160;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:160;

(c) an amino acid sequence of a fragment of SEQ ID NO: 160, the fragment having the activity of 1-deoxyxylulose-5-phosphate synthase (DXPS);

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1185-1610 of SEQ ID NO:157 or a complement thereof, wherein the polypeptide has the activity of DXPS;

(e) a conservatively modified variant of SEQ ID NO:160.

4. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 390 of SEQ ID NO:178;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:178;

(c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 178, the fragment having the activity of acetyl-CoA acetyltransferase;

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1-1170 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetyl-CoA acetyltransferase; and

(e) a conservatively modified variant of SEQ ID NO:178.

5. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence shown as residues 1 to 240 of SEQ ID NO:179;

(b) at least 30 contiguous amino acid residues of SEQ ID NO:179;

(c) an amino acid sequence of a fragment of a polypeptide of SEQ ID NO: 179, the fragment having the activity of acetoacetyl-CoA reductase;

(d) an amino acid sequence of a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a hybridization probe comprising at least 30 consecutive nucleotides spanning positions 1258-1980 of SEQ ID NO:177 or a complement thereof, wherein the polypeptide has the activity of acetoacetyl-CoA reductase; and

(e) a conservatively modified variant of SEQ ID NO:179.

6. An isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, variants of SEQ ID NO:42 containing one or more

substitutions according to the *Paracoccus* sp. strain R1534 codon usage table, fragments of SEQ ID NO:42 that encode a polypeptide having an activity selected from the group consisting of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), isopentenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase), mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:42, or the complement of SEQ ID NO:42, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of HMG-CoA reductase, isopentenyl diphosphate isomerase, HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and diphosphomevalonate decarboxylase.

7. An isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:157, variants of SEQ ID NO:157 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table, fragments of SEQ ID NO:157 that encode a polypeptide having farnesyl diphosphate (FPP) synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity or a polypeptide having the activity of XseB, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:157, or the complement of SEQ ID NO:157, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of FPP synthase activity, 1-deoxy-D-xylulose 5-phosphate synthase activity, and the activity of XseB.

8. An isolated polynucleotide sequence comprising a polynucleotide sequence selected from the group consisting of the nucleotide sequence of SEQ ID NO:177, variants of SEQ ID NO:177 containing one or more substitutions according to the *Paracoccus* sp. strain R1534 codon usage table, fragments of SEQ ID NO:177 that encode a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase, and polynucleotide sequences that hybridize under stringent conditions to a hybridization probe the nucleotide sequence of which consists of at least 30 contiguous nucleotides of SEQ ID NO:177, or the complement of SEQ ID NO:177, which polynucleotide encodes a polypeptide having an activity selected from the group consisting of acetyl-CoA acetyltransferase and acetoacetyl-CoA reductase.

9. An isolated polynucleotide sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:42, SEQ ID NO:157, SEQ ID NO:177, and combinations thereof.

10. An expression vector comprising the polynucleotide sequence according to claim 6, 7, 8 or 9.

11. An expression vector selected from the group consisting of pBBR-K-mev-op16-1, pBBR-K-mev-op16-2, pDS-*mvaA*, pDS-*idi*, pDS-*hcs*, pDS-*mvk*, pDS-*pmk*, pDS-*mvd*, pDS-His–*mvaA*, pDS-His–*idi*, pDS-His-*hcs*, pDS-His–*mvk*, pDS-His–*pmk*, pDS-His-*mvd*, pBBR-K-Zea4, pBBR-K-Zea4-up, pBBR-K-Zea4-down, pBBR-K-P*crtE-crtE*-3, pBBR-tK-P*crtE-mva*A, pBBR-tK-P*crtE-idi*, pBBR-tK-P*crtE-hcs*, pBBR-tK-P*crtE-mvk*, pBBR-tK-P*crtE-pmk*, pBBR-tK-P*crtE-mvd*, pBBR-K-P*crtE-mvaA-crtE*-3, pDS-His-*phaA*, pBBR-K-P*crtE-crtW*, pBBR-K-P*crtE-crtWZ*, pBBR-K-P*crtE-crtZW*, and combinations thereof.

12. A cultured cell comprising the polynucleotide sequence according to claim 6, 7, 8 or 9, or an expression vector according to claim 10 or 11, or a progeny of the cell, wherein the cell expresses a polypeptide encoded by the polynucleotide sequence.

13. A method of producing a carotenoid comprising culturing a cell according to claim 12 under conditions permitting expression of a polypeptide encoded by the polynucleotide sequence, and isolating the carotenoid from the cell or the medium of the cell.

14. A method of making a carotenoid-producing cell comprising:
(a) introducing into a cell a polynucleotide sequence encoding an enzyme in the mevalonate pathway, which enzyme is expressed in the cell; and
(b) selecting a cell containing the polynucleotide sequence of step (a) that produces a carotenoid at a level that is about 1.1-1,000 times the level of the carotenoid produced by the cell before introduction of the polynucleotide sequence.

15. A method for engineering a bacterium to produce an isoprenoid compound comprising:
(a) culturing a parent bacterium in a medium under conditions permitting expression of an isoprenoid compound, and selecting a mutant bacterium from the culture medium that produces about 1.1-1,000 times more of an isoprenoid compound than the parent bacterium;
(b) introducing into the mutant bacterium an expression vector comprising a polynucleotide sequence represented by SEQ ID NO:42 operably linked to an expression control sequence; and
(c) selecting a bacterium that contains the expression vector and produces at least about 1.1 times more of an isoprenoid compound than the mutant in step (a).

16. A microorganism of the genus *Paracoccus*, which microorganism has the following characteristics:

(i) a sequence similiarity to SEQ ID NO:12 of >97% using a similarity matrix obtained from a homology calculation using GeneCompar v. 2.0 software with a gap penalty of 0%;

a homology to strain R-1512, R1534, R114 or R-1506 of >70% using DNA:DNA hybridization at 81.5°C;

a G+C content of its genomic DNA that varies less than 1% from the G+C content of the genomic DNA of R114, R-1512, R1534, and R-1506; and

an average DNA fingerprint that clusters at about 58% similarity to strains R-1512, R1534, R114 and R-1506 using the AFLP procedure of Example 2, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966);

(ii) 18:1w7c comprising at least about 75% of the total fatty acids of the cell membranes;

an inability to use adonitol, i-erythritol, gentiobiose, β-methylglucoside, D-sorbitol, xylitol and quinic acid as carbon sources for growth; and

an ability to use L-asparagine and L-aspartic acid as carbon sources for growth, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966); or

(iii) an ability to grow at 40°C;

an ability to grow in a medium having 8% NaCl;

an ability to grow in a medium having a pH of 9.1; and

a yellow-orange colony pigmentation, with the proviso that the microorganism is not *Paracoccus* sp. (MBIC3966).

SEQUENCE LISTING

<110> BERRY, Alan
BRETZEL, Werner
HUMBELIN, Markus
LOPEZ-ULIBARRI, Rual
MAYER, Anne
YELISEEV, Alexei

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acc aag gcc gcc gag gcc gtg cag ggc tgg atc ctg tcg gaa tac ccg      3068
Thr Lys Ala Ala Glu Ala Val Gln Gly Trp Ile Leu Ser Glu Tyr Pro
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Met Leu Ala Tyr Ser Thr Ile Ser Gly Asn Leu Cys Thr Asp Lys Lys
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gag gtc gag atc ccg cgc aag atc ctg acc cgc gtg ctg cgc acc agc      3212
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gcc gag aag atg gtc cgc ctg aac tac gag aag aac tat gtc ggg ggt      3260
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Arg Ser Ile Ala Leu Arg Ala His Asp Gly Ala Ala Ala Thr Ala Ala
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Gly His Asn Met Val Thr Lys Ala Ala Glu Ala Val Gln Gly Trp Ile
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Cys Thr Asp Lys Lys Ala Ser Ala Val Asn Gly Ile Leu Gly Arg Gly
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Lys Tyr Ala Val Ala Glu Val Glu Ile Pro Arg Lys Ile Leu Thr Arg
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Val Leu Arg Thr Ser Ala Glu Lys Met Val Arg Leu Asn Tyr Glu Lys
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Asn Tyr Val Gly Gly Thr Leu Ala Gly Ser Leu Arg Ser Ala Asn Ala
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His Phe Ala Asn Met Leu Leu Gly Phe Tyr Leu Ala Thr Gly Gln Asp
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Ala Ala Asn Ile Ile Glu Ala Ser Gln Gly Phe Val His Cys Glu Ala
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Arg Gly Glu Asp Leu Tyr Phe Ser Cys Thr Leu Pro Asn Leu Ile Met
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Gly Ser Val Gly Ala Gly Lys Gly Ile Pro Ser Ile Glu Glu Asn Leu
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Leu Thr His Arg Ala Leu Pro Glu Val Asp Phe Asp Ala Ile Asp Thr
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tcc atg acc ggc ggc acc ggc gag gag atc gag cgc atc aac cgc aac     3895
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Arg Pro Ala Leu Ala Ala His Asp Gly Thr Ser Val Leu Ile Ala Ser
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Gly Gly Ile Arg Asn Gly Val Asp Met Ala Lys Cys Val Ile Leu Gly
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Val Leu Leu Lys Glu Val Gly Cys Gly Leu Ser Ala Ala Asp Ile Ala
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Lys Val Leu Thr His Pro Asp Asp Leu Ala Val Tyr Ala Leu Ala Ser
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Ala Met His His Leu Pro Arg Pro Gly Glu Leu Gly Ser Arg Thr Glu
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Gln Arg Phe Asp Arg Val Arg Phe Cys Glu Arg Leu Lys His Gly Lys
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ccgttcggcc tgcgctatgc gggctacaag accccgacag ccgaggtgct gcgccttgtg 7560

gccgatcgga tggcgggcaa cgaggccgct ttcgacgcgc tctactcccg gatgggcgca 7620

agcgcagatg ccgcgatccg cgcggcgcaa gggctggact gggctgcatt ccacgacgcg 7680

ctgaacgaat accagcgcct gatggagcag ctgggcgtgt ccgacgacac gctggacgcg 7740

atcatccgcg aggcgcgcga cgcgggcgcc gcagtcgcca agatctccgg ctcggggctg 7800

ggggattgcg tgctggcact gggcgaccag cccaagggtt tcgtgcccgc aagcattgcc 7860

gagaagggac ttgttttcg atg act gat gcc gtc cgc gac atg atc gcc cgt 7912

```
                     Met Thr Asp Ala Val Arg Asp Met Ile Ala Arg
                     1               5                   10
```

```
gcc atg gcg ggc gcg acc gac atc cga gca gcc gag gct tat gcg ccc        7960
Ala Met Ala Gly Ala Thr Asp Ile Arg Ala Ala Glu Ala Tyr Ala Pro
            15                  20                  25

agc aac atc gcg ctg tcg aaa tac tgg ggc aag cgc gac gcc gcg cgg        8008
Ser Asn Ile Ala Leu Ser Lys Tyr Trp Gly Lys Arg Asp Ala Ala Arg
        30                  35                  40

aac ctt ccg ctg aac agc tcc gtc tcg atc tcg ttg gcg aac tgg ggc        8056
Asn Leu Pro Leu Asn Ser Ser Val Ser Ile Ser Leu Ala Asn Trp Gly
    45                  50                  55

tct cat acg cgg gtc gag ggg tcc ggc acg ggc cac gac gag gtg cat        8104
Ser His Thr Arg Val Glu Gly Ser Gly Thr Gly His Asp Glu Val His
60                  65                  70                  75

cac aac ggc acg ctg ctg gat ccg ggc gac gcc ttc gcg cgc cgc gcg        8152
His Asn Gly Thr Leu Leu Asp Pro Gly Asp Ala Phe Ala Arg Arg Ala
                80                  85                  90

ttg gca ttc gct gac ctg ttc cgg ggg ggg agg cac ctg ccg ctg cgg        8200
Leu Ala Phe Ala Asp Leu Phe Arg Gly Gly Arg His Leu Pro Leu Arg
            95                  100                 105

atc acg acg cag aac tcg atc ccg acg gcg gcg ggg ctt gcc tcg tcg        8248
Ile Thr Thr Gln Asn Ser Ile Pro Thr Ala Ala Gly Leu Ala Ser Ser
        110                 115                 120

gcc tcg ggg ttc gcg gcg ctg acc cgt gcg ctg gcg ggg gcg ttc ggg        8296
Ala Ser Gly Phe Ala Ala Leu Thr Arg Ala Leu Ala Gly Ala Phe Gly
    125                 130                 135

ctg gat ctg gac gac acg gat ctg agc cgc atc gcc cgg atc ggc agt        8344
Leu Asp Leu Asp Asp Thr Asp Leu Ser Arg Ile Ala Arg Ile Gly Ser
140                 145                 150                 155

ggc agc gcc gcc cgc tcg atc tgg cac ggc ttc gtc cgc tgg aac cgg        8392
Gly Ser Ala Ala Arg Ser Ile Trp His Gly Phe Val Arg Trp Asn Arg
                160                 165                 170

ggc gag gcc gag gat ggg cat gac agc cac ggc gtc ccg ctg gac ctg        8440
Gly Glu Ala Glu Asp Gly His Asp Ser His Gly Val Pro Leu Asp Leu
            175                 180                 185

cgc tgg ccc ggc ttc cgc atc gcg atc gtg gcc gtg gac aag ggg ccc        8488
Arg Trp Pro Gly Phe Arg Ile Ala Ile Val Ala Val Asp Lys Gly Pro
        190                 195                 200

aag cct ttc agt tcg cgc gac ggc atg aac cac acg gtc gag acc agc        8536
Lys Pro Phe Ser Ser Arg Asp Gly Met Asn His Thr Val Glu Thr Ser
    205                 210                 215

ccg ctg ttc ccg ccc tgg cct gcg cag gcg gaa gcg gat tgc cgc gtc        8584
Pro Leu Phe Pro Pro Trp Pro Ala Gln Ala Glu Ala Asp Cys Arg Val
220                 225                 230                 235
```

atc gag gat gcg atc gcc gcc cgc gac atg gcc gcc ctg ggt ccg cgg 8632
Ile Glu Asp Ala Ile Ala Ala Arg Asp Met Ala Ala Leu Gly Pro Arg
240 245 250

gtc gag gcg aac gcc ctt gcg atg cac gcc acg atg atg gcc gcg cgc 8680
Val Glu Ala Asn Ala Leu Ala Met His Ala Thr Met Met Ala Ala Arg
255 260 265

ccg ccg ctc tgc tac ctg acg ggc ggc agc tgg cag gtg ctg gaa cgc 8728
Pro Pro Leu Cys Tyr Leu Thr Gly Gly Ser Trp Gln Val Leu Glu Arg
270 275 280

ctg tgg cag gcc cgc gcg gac ggg ctt gcg gcc ttt gcg acg atg gat 8776
Leu Trp Gln Ala Arg Ala Asp Gly Leu Ala Ala Phe Ala Thr Met Asp
285 290 295

gcc ggc ccg aac gtc aag ctg atc ttc gag gaa agc agc gcc gcc gac 8824
Ala Gly Pro Asn Val Lys Leu Ile Phe Glu Glu Ser Ser Ala Ala Asp
300 305 310 315

gtg ctg tac ctg ttc ccc gac gcc agc ctg atc gcg ccg ttc gag ggg 8872
Val Leu Tyr Leu Phe Pro Asp Ala Ser Leu Ile Ala Pro Phe Glu Gly
320 325 330

cgt tga acgcgtaaga cgaccactgg gtaaggttct gccgcgcgtg gtctcgactg 8928
Arg

cctgcaaaga ggtgcttgag ttgctgcgtg actgcggcgg ccgacttcgt gggacttgcc 8988

cgccacgctg acgcgctgga aacgcgcccg cggattacga ccgcgtcatt gccctgaacc 9048

aatttcccgt cggtcgac 9066

<210> 53

<211> 332

<212> PRT

<213> Paracoccus sp. R114

<400> 53

Met Thr Asp Ala Val Arg Asp Met Ile Ala Arg Ala Met Ala Gly Ala
1 5 10 15

Thr Asp Ile Arg Ala Ala Glu Ala Tyr Ala Pro Ser Asn Ile Ala Leu
20 25 30

Ser Lys Tyr Trp Gly Lys Arg Asp Ala Ala Arg Asn Leu Pro Leu Asn
35 40 45

```
Ser Ser Val Ser Ile Ser Leu Ala Asn Trp Gly Ser His Thr Arg Val
    50                  55                  60

Glu Gly Ser Gly Thr Gly His Asp Glu Val His His Asn Gly Thr Leu
65                  70                  75                  80

Leu Asp Pro Gly Asp Ala Phe Ala Arg Arg Ala Leu Ala Phe Ala Asp
                85                  90                  95

Leu Phe Arg Gly Gly Arg His Leu Pro Leu Arg Ile Thr Thr Gln Asn
            100                 105                 110

Ser Ile Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe Ala
        115                 120                 125

Ala Leu Thr Arg Ala Leu Ala Gly Ala Phe Gly Leu Asp Leu Asp Asp
    130                 135                 140

Thr Asp Leu Ser Arg Ile Ala Arg Ile Gly Ser Gly Ser Ala Ala Arg
145                 150                 155                 160

Ser Ile Trp His Gly Phe Val Arg Trp Asn Arg Gly Glu Ala Glu Asp
                165                 170                 175

Gly His Asp Ser His Gly Val Pro Leu Asp Leu Arg Trp Pro Gly Phe
            180                 185                 190

Arg Ile Ala Ile Val Ala Val Asp Lys Gly Pro Lys Pro Phe Ser Ser
        195                 200                 205

Arg Asp Gly Met Asn His Thr Val Glu Thr Ser Pro Leu Phe Pro Pro
    210                 215                 220

Trp Pro Ala Gln Ala Glu Ala Asp Cys Arg Val Ile Glu Asp Ala Ile
225                 230                 235                 240

Ala Ala Arg Asp Met Ala Ala Leu Gly Pro Arg Val Glu Ala Asn Ala
                245                 250                 255

Leu Ala Met His Ala Thr Met Met Ala Ala Arg Pro Pro Leu Cys Tyr
            260                 265                 270
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Leu Thr Gly Gly Ser Trp Gln Val Leu Glu Arg Leu Trp Gln Ala Arg
275 280 285

Ala Asp Gly Leu Ala Ala Phe Ala Thr Met Asp Ala Gly Pro Asn Val
290 295 300

Lys Leu Ile Phe Glu Glu Ser Ser Ala Ala Asp Val Leu Tyr Leu Phe
305 310 315 320

Pro Asp Ala Ser Leu Ile Ala Pro Phe Glu Gly Arg
325 330

<210> 54

<211> 353

<212> PRT

<213> Streptomyces sp. strain CL190

<400> 54

Met Thr Glu Thr His Ala Ile Ala Gly Val Pro Met Arg Trp Val Gly
1 5 10 15

Pro Leu Arg Ile Ser Gly Asn Val Ala Glu Thr Glu Thr Gln Val Pro
20 25 30

Leu Ala Thr Tyr Glu Ser Pro Leu Trp Pro Ser Val Gly Arg Gly Ala
35 40 45

Lys Val Ser Arg Leu Thr Glu Lys Gly Ile Val Ala Thr Leu Val Asp
50 55 60

Glu Arg Met Thr Arg Ser Val Ile Val Glu Ala Thr Asp Ala Gln Thr
65 70 75 80

Ala Tyr Met Ala Ala Gln Thr Ile His Ala Arg Ile Asp Glu Leu Arg
85 90 95

Glu Val Val Arg Gly Cys Ser Arg Phe Ala Gln Leu Ile Asn Ile Lys
100 105 110

His Glu Ile Asn Ala Asn Leu Leu Phe Ile Arg Phe Glu Phe Thr Thr
115 120 125

Gly Asp Ala Ser Gly His Asn Met Ala Thr Leu Ala Ser Asp Val Leu
130 135 140

Leu Gly His Leu Leu Glu Thr Ile Pro Gly Ile Ser Tyr Gly Ser Ile

145 150 155 160

Ser Gly Asn Tyr Cys Thr Asp Lys Lys Ala Thr Ala Ile Asn Gly Ile
165 170 175

Leu Gly Arg Gly Lys Asn Val Ile Thr Glu Leu Leu Val Pro Arg Asp
180 185 190

Val Val Glu Asn Asn Leu His Thr Thr Ala Ala Lys Ile Val Glu Leu
195 200 205

Asn Ile Arg Lys Asn Leu Leu Gly Thr Leu Leu Ala Gly Gly Ile Arg
210 215 220

Ser Ala Asn Ala His Phe Ala Asn Met Leu Leu Gly Phe Tyr Leu Ala
225 230 235 240

Thr Gly Gln Asp Ala Ala Asn Ile Val Glu Gly Ser Gln Gly Val Val
245 250 255

Met Ala Glu Asp Arg Asp Gly Asp Leu Tyr Phe Ala Cys Thr Leu Pro
260 265 270

Asn Leu Ile Val Gly Thr Val Gly Asn Gly Lys Gly Leu Gly Phe Val
275 280 285

Glu Thr Asn Leu Ala Arg Leu Gly Cys Arg Ala Asp Arg Glu Pro Gly
290 295 300

Glu Asn Ala Arg Arg Leu Ala Val Ile Ala Ala Ala Thr Val Leu Cys
305 310 315 320

Gly Glu Leu Ser Leu Leu Ala Ala Gln Thr Asn Pro Gly Glu Leu Met
325 330 335

Arg Ala His Val Gln Leu Glu Arg Asp Asn Lys Thr Ala Lys Val Gly
340 345 350

Ala

<210> 55

<211> 353

<212> PRT

<213> Streptomyces griseolosporeus

<400> 55

Met Thr Glu Ala His Ala Thr Ala Gly Val Pro Met Arg Trp Val Gly
1 5 10 15

Pro Val Arg Ile Ser Gly Asn Val Ala Thr Ile Glu Thr Gln Val Pro

20 25 30

Leu Ala Thr Tyr Glu Ser Pro Leu Trp Pro Ser Val Gly Arg Gly Ala
35 40 45

Lys Val Ser Arg Leu Thr Glu Lys Gly Ile Val Ala Thr Leu Val Asp
50 55 60

Glu Arg Met Thr Arg Ser Val Leu Val Glu Ala Thr Asp Ala Leu Thr
65 70 75 80

Ala Leu Ser Ala Ala Arg Thr Ile Glu Ala Arg Ile Asp Glu Leu Arg
85 90 95

Glu Leu Val Arg Gly Cys Ser Arg Phe Ala Gln Leu Ile Gly Ile Arg
100 105 110

His Glu Ile Thr Gly Asn Leu Leu Phe Val Arg Phe Glu Phe Ser Thr
115 120 125

Gly Asp Ala Ser Gly His Asn Met Ala Thr Leu Ala Ser Asp Val Leu
130 135 140

Leu Gln His Leu Leu Glu Thr Val Pro Gly Ile Ser Tyr Gly Ser Ile
145 150 155 160

Ser Gly Asn Tyr Cys Thr Asp Lys Lys Ala Thr Ala Ile Asn Gly Ile
165 170 175

Leu Gly Arg Gly Lys Asn Val Val Thr Glu Leu Leu Val Pro Arg Asp
180 185 190

Val Val Ala Asp Val Leu Asn Thr Thr Ala Ala Lys Ile Ala Glu Leu
195 200 205

Asn Leu Arg Lys Asn Leu Leu Gly Thr Leu Leu Ala Gly Gly Ile Arg
210 215 220

Ser Ala Asn Ala His Tyr Ala Asn Met Leu Leu Ala Phe Tyr Leu Ala
225 230 235 240

Thr Gly Gln Asp Ala Ala Asn Ile Val Glu Gly Ser Gln Gly Val Val
245 250 255

Thr Ala Glu Asp Arg Asp Gly Asp Leu Tyr Leu Ala Cys Thr Leu Pro
260 265 270

Asn Leu Ile Val Gly Thr Val Gly Asn Gly Lys Gly Leu Gly Phe Val
275 280 285

Glu Thr Asn Leu Asn Arg Leu Gly Cys Arg Ala Asp Arg Glu Pro Gly
290 295 300

Glu Asn Ala Arg Arg Leu Ala Val Ile Ala Ala Ala Thr Val Leu Cys
305 310 315 320

Gly Glu Leu Ser Leu Leu Ala Ala Gln Thr Asn Pro Gly Glu Leu Met

325 330 335

Arg Ala His Val Gln Leu Glu Arg Gly His Thr Thr Ala Lys Ala Gly
340 345 350

Val

<210> 56

<211> 353

<212> PRT

<213> Streptomyces sp. strain KO-3899

<400> 56

Met Thr Asp Thr His Ala Ile Ala Met Val Pro Met Lys Trp Val Gly
1 5 10 15

Pro Leu Arg Ile Ser Gly Asn Val Ala Thr Thr Glu Thr His Val Pro
20 25 30

Leu Ala Thr Tyr Glu Thr Pro Leu Trp Pro Ser Val Gly Arg Gly Ala
35 40 45

Lys Val Ser Met Leu Ser Glu Arg Gly Ile Ala Ala Thr Leu Val Asp
50 55 60

Glu Arg Met Thr Arg Ser Val Leu Val Glu Ala Thr Asp Ala Gln Thr
65 70 75 80

Ala Tyr Thr Ala Ala Arg Ala Ile Glu Ala Arg Ile Glu Glu Leu Arg
85 90 95

Ala Val Val Arg Thr Cys Ser Arg Phe Ala Glu Leu Leu Gln Val Arg
100 105 110

His Glu Ile Ala Gly Asn Leu Leu Phe Val Arg Phe Glu Phe Ser Thr
115 120 125

Arg Arg Pro Ser Gly His Asn Met Ala Thr Leu Ala Ser Asp Ala Leu
130 135 140

Leu Ala His Leu Leu Gln Thr Ile Pro Gly Ile Ser Tyr Gly Ser Ile
145 150 155 160

Ser Gly Asn Tyr Cys Thr Asp Lys Lys Ala Thr Ala Ile Asn Gly Ile
165 170 175

Leu Gly Arg Gly Lys Asn Val Val Thr Glu Leu Val Val Pro Arg Glu
180 185 190

Val Val Glu Arg Val Leu His Thr Thr Ala Ala Lys Ile Val Glu Leu

195 200 205

Asn Ile Arg Lys Asn Leu Leu Gly Thr Leu Leu Ala Gly Gly Ile Arg
210 215 220

Ser Ala Asn Ala His Tyr Ala Asn Met Leu Leu Gly Phe Tyr Leu Ala
225 230 235 240

Thr Gly Gln Asp Ala Ala Asn Ile Val Glu Gly Ser Gln Gly Val Thr
245 250 255

Leu Ala Glu Asp Arg Asp Gly Asp Leu Tyr Phe Ser Cys Asn Leu Pro
260 265 270

Asn Leu Ile Val Gly Thr Val Gly Asn Gly Lys Gly Leu Glu Phe Val
275 280 285

Glu Thr Asn Leu Asn Arg Leu Gly Cys Arg Glu Asp Arg Ala Pro Gly
290 295 300

Glu Asn Ala Arg Arg Leu Ala Val Ile Ala Ala Ala Thr Val Leu Cys
305 310 315 320

Gly Glu Leu Ser Leu Leu Ala Ala Gln Thr Asn Pro Gly Glu Leu Met
325 330 335

Arg Ala His Val Glu Leu Glu Arg Asp Asn Thr Thr Ala Glu Val Gly
340 345 350

Val

<210> 57

<211> 347

<212> PRT

<213> Erwinia herbicola

<400> 57

Met Lys Asp Glu Arg Leu Val Gln Arg Lys Asn Asp His Leu Asp Ile
1 5 10 15

Val Leu Asp Pro Arg Arg Ala Val Thr Gln Ala Ser Ala Gly Phe Glu
20 25 30

Arg Trp Arg Phe Thr His Cys Ala Leu Pro Glu Leu Asn Phe Ser Asp
35 40 45

Ile Thr Leu Glu Thr Thr Phe Leu Asn Arg Gln Leu Gln Ala Pro Leu
50 55 60

Leu Ile Ser Ser Met Thr Gly Gly Val Glu Arg Ser Arg His Ile Asn

65 70 75 80

Arg His Leu Ala Glu Ala Ala Gln Val Leu Lys Ile Ala Met Gly Val
85 90 95

Gly Ser Gln Arg Val Ala Ile Glu Ser Asp Ala Gly Leu Gly Leu Asp
100 105 110

Lys Thr Leu Arg Gln Leu Ala Pro Asp Val Pro Leu Leu Ala Asn Leu
115 120 125

Gly Ala Ala Gln Leu Thr Gly Arg Lys Gly Ile Asp Tyr Ala Arg Arg
130 135 140

Ala Val Glu Met Ile Glu Ala Asp Ala Leu Ile Val His Leu Asn Pro
145 150 155 160

Leu Gln Glu Ala Leu Gln Pro Gly Gly Asp Arg Asp Trp Arg Gly Arg
165 170 175

Leu Ala Ala Ile Glu Thr Leu Val Arg Glu Leu Pro Val Pro Leu Val
180 185 190

Val Lys Glu Val Gly Ala Gly Ile Ser Arg Thr Val Ala Gly Gln Leu
195 200 205

Ile Asp Ala Gly Val Thr Val Ile Asp Val Ala Gly Ala Gly Gly Thr
210 215 220

Ser Trp Ala Ala Val Glu Gly Glu Arg Ala Ala Thr Glu Gln Gln Arg
225 230 235 240

Ser Val Ala Asn Val Phe Ala Asp Trp Gly Ile Pro Thr Ala Glu Ala
245 250 255

Leu Val Asp Ile Ala Glu Ala Trp Pro Gln Met Pro Leu Ile Ala Ser
260 265 270

Gly Gly Ile Lys Asn Gly Val Asp Ala Ala Lys Ala Leu Arg Leu Gly
275 280 285

Ala Cys Met Val Gly Gln Ala Ala Ala Val Leu Gly Ser Ala Gly Val
290 295 300

Ser Thr Glu Lys Val Ile Asp His Phe Asn Val Ile Ile Glu Gln Leu
305 310 315 320

Arg Val Ala Cys Phe Cys Thr Gly Ser Arg Ser Leu Ser Asp Leu Lys
325 330 335

Gln Ala Asp Ile Arg Tyr Val Arg Asp Thr Pro
340 345

<210> 58

<211> 360

<212> PRT

<213> Borrelia burgdorferi

<400> 58

Met Met Asp Thr Glu Phe Met Gly Ile Glu Pro Asn Ile Leu Glu Asn
1 5 10 15

Lys Lys Arg His Ile Glu Ile Cys Leu Asn Lys Asn Asp Val Lys Gly
20 25 30

Gly Cys Asn Phe Leu Lys Phe Ile Lys Leu Lys His Asn Ala Leu Ser
35 40 45

Asp Phe Asn Phe Ser Glu Ile Asn Ile Lys Glu Glu Ile Phe Gly Tyr
50 55 60

Asn Ile Ser Met Pro Val Phe Ile Ser Ser Met Thr Gly Gly Ser Lys
65 70 75 80

Glu Gly Asn Asp Phe Asn Lys Ser Leu Val Arg Ile Ala Asn Tyr Leu
85 90 95

Lys Ile Pro Ile Gly Leu Gly Ser Phe Lys Leu Leu Phe Lys Tyr Pro
100 105 110

Glu Tyr Ile Arg Asp Phe Thr Leu Lys Arg Tyr Ala His Asn Ile Pro
115 120 125

Leu Phe Ala Asn Val Gly Ala Val Gln Ile Val Glu Phe Gly Ile Ser
130 135 140

Lys Ile Ala Glu Met Ile Lys Arg Leu Glu Val Asp Ala Ile Ile Val
145 150 155 160

His Leu Asn Ala Gly Gln Glu Leu Met Lys Val Asp Gly Asp Arg Asn
165 170 175

Phe Lys Gly Ile Arg Glu Ser Ile Ala Lys Leu Ser Asp Phe Leu Ser
180 185 190

Val Pro Leu Ile Val Lys Glu Thr Gly Phe Gly Ile Ser Pro Lys Asp
195 200 205

Val Lys Glu Leu Phe Ser Leu Gly Ala Ser Tyr Val Asp Leu Ala Gly
210 215 220

Ser Gly Gly Thr Asn Trp Ile Leu Val Glu Gly Met Lys Ser Asn Asn
225 230 235 240

Leu Asn Ile Ala Ser Cys Phe Ser Asp Trp Gly Ile Pro Ser Val Phe
245 250 255

Thr Leu Leu Ser Ile Asp Asp Ser Leu Lys Ala Asn Ile Phe Ala Ser

260 265 270

Gly Gly Tyr Glu Thr Gly Met Asp Ile Ala Lys Gly Ile Ala Leu Gly
275 280 285

Ala Arg Leu Ile Gly Val Ala Ala Val Val Leu Arg Ala Phe Tyr Asp
290 295 300

Ser Gly Glu Asp Ala Val Phe Gly Leu Phe Ser Asp Tyr Glu His Ile
305 310 315 320

Leu Lys Met Ser Met Phe Leu Ser Gly Ser Lys Ser Leu Leu Glu Phe
325 330 335

Arg Asn Asn Lys Tyr Phe Leu Ser Ser Tyr Leu Leu Asp Glu Leu Gly
340 345 350

Val Phe Lys Gln Phe Tyr Gly Thr
355 360

<210> 59

<211> 349

<212> PRT

<213> Synechocystis sp. PCC 6803

<400> 59

Met Asp Ser Thr Pro His Arg Lys Ser Asp His Ile Arg Ile Val Leu
1 5 10 15

Glu Glu Asp Val Val Gly Lys Gly Ile Ser Thr Gly Phe Glu Arg Leu
20 25 30

Met Leu Glu His Cys Ala Leu Pro Ala Val Asp Leu Asp Ala Val Asp
35 40 45

Leu Gly Leu Thr Leu Trp Gly Lys Ser Leu Thr Tyr Pro Trp Leu Ile
50 55 60

Ser Ser Met Thr Gly Gly Thr Pro Glu Ala Lys Gln Ile Asn Leu Phe
65 70 75 80

Leu Ala Glu Val Ala Gln Ala Leu Gly Ile Ala Met Gly Leu Gly Ser
85 90 95

Gln Arg Ala Ala Ile Glu Asn Pro Asp Leu Ala Phe Thr Tyr Gln Val
100 105 110

Arg Ser Val Ala Pro Asp Ile Leu Leu Phe Ala Asn Leu Gly Leu Val
115 120 125

Gln Leu Asn Tyr Gly Tyr Gly Leu Glu Gln Ala Gln Arg Ala Val Asp

130 135 140

Met Ile Glu Ala Asp Ala Leu Ile Leu His Leu Asn Pro Leu Gln Glu
145 150 155 160

Ala Val Gln Pro Asp Gly Asp Arg Leu Trp Ser Gly Leu Trp Ser Lys
165 170 175

Leu Glu Ala Leu Val Glu Ala Leu Glu Val Pro Val Ile Val Lys Glu
180 185 190

Val Gly Asn Gly Ile Ser Gly Pro Val Ala Lys Arg Leu Gln Glu Cys
195 200 205

Gly Val Gly Ala Ile Asp Val Ala Gly Ala Gly Gly Thr Ser Trp Ser
210 215 220

Glu Val Glu Ala His Arg Gln Thr Asp Arg Gln Ala Lys Glu Val Ala
225 230 235 240

His Asn Phe Ala Asp Trp Gly Leu Pro Thr Ala Trp Ser Leu Gln Gln
245 250 255

Val Val Gln Asn Thr Glu Gln Ile Leu Val Phe Ala Ser Gly Gly Ile
260 265 270

Arg Ser Gly Ile Asp Gly Ala Lys Ala Ile Ala Leu Gly Ala Thr Leu
275 280 285

Val Gly Ser Ala Ala Pro Val Leu Ala Glu Ala Lys Ile Asn Ala Gln
290 295 300

Arg Val Tyr Asp His Tyr Gln Ala Arg Leu Arg Glu Leu Gln Ile Ala
305 310 315 320

Ala Phe Cys Cys Asp Ala Ala Asn Leu Thr Gln Leu Ala Gln Val Pro
325 330 335

Leu Trp Asp Arg Gln Ser Gly Gln Arg Leu Thr Lys Pro
340 345

<210> 60

<211> 361

<212> PRT

<213> Streptomyces sp. CL190

<400> 60

Met Thr Ser Ala Gln Arg Lys Asp Asp His Val Arg Leu Ala Ile Glu
1 5 10 15

Gln His Asn Ala His Ser Gly Arg Asn Gln Asp Asp Val Ser Phe Val

```
            20                  25                  30

His His Ala Leu Ala Gly Ile Asp Arg Pro Asp Val Ser Leu Ala Thr
        35                  40                  45

Ser Phe Ala Gly Ile Ser Trp Gln Val Pro Ile Tyr Ile Asn Ala Met
    50                  55                  60

Thr Gly Gly Ser Glu Lys Thr Gly Leu Ile Asn Arg Asp Leu Ala Thr
65                  70                  75                  80

Ala Ala Arg Glu Thr Gly Val Pro Ile Ala Ser Gly Ser Met Asn Ala
                85                  90                  95

Tyr Ile Lys Asp Pro Cys Ala Asp Thr Phe Arg Val Leu Arg Asp Glu
            100                 105                 110

Asn Pro Asn Gly Phe Val Ile Ala Asn Ile Asn Ala Thr Thr Thr Val
        115                 120                 125

Asp Asn Ala Gln Arg Ala Ile Asp Leu Ile Glu Ala Asn Ala Leu Gln
    130                 135                 140

Ile His Ile Asn Thr Ala Gln Glu Thr Pro Met Pro Glu Gly Asp Arg
145                 150                 155                 160

Ser Phe Ala Ser Trp Val Pro Gln Ile Glu Lys Ile Ala Ala Ala Val
                165                 170                 175

Asp Ile Pro Val Ile Val Lys Glu Val Gly Asn Gly Leu Ser Arg Gln
            180                 185                 190

Thr Ile Leu Leu Leu Ala Asp Leu Gly Val Gln Ala Ala Asp Val Ser
        195                 200                 205

Gly Arg Gly Gly Thr Asp Phe Ala Arg Ile Glu Asn Gly Arg Arg Glu
    210                 215                 220

Leu Gly Asp Tyr Ala Phe Leu His Gly Trp Gly Gln Ser Thr Ala Ala
225                 230                 235                 240

Cys Leu Leu Asp Ala Gln Asp Ile Ser Leu Pro Val Leu Ala Ser Gly
                245                 250                 255

Gly Val Arg His Pro Leu Asp Val Val Arg Ala Leu Ala Leu Gly Ala
            260                 265                 270

Arg Ala Val Gly Ser Ser Ala Gly Phe Leu Arg Thr Leu Met Asp Asp
        275                 280                 285

Gly Val Asp Ala Leu Ile Thr Lys Leu Thr Thr Trp Leu Asp Gln Leu
    290                 295                 300

Ala Ala Leu Gln Thr Met Leu Gly Ala Arg Thr Pro Ala Asp Leu Thr
305                 310                 315                 320

Arg Cys Asp Val Leu Leu His Gly Glu Leu Arg Asp Phe Cys Ala Asp
```

325 330 335

Arg Gly Ile Asp Thr Arg Arg Leu Ala Gln Arg Ser Ser Ser Ile Glu
340 345 350

Ala Leu Gln Thr Thr Gly Ser Thr Arg
355 360

<210> 61

<211> 364

<212> PRT

<213> Streptomyces griseolosporeus

<400> 61

Met Ser Ser Ala Gln Arg Lys Asp Asp His Val Arg Leu Ala Thr Glu
1 5 10 15

Gln Gln Arg Ala His Ser Gly Arg Asn Gln Phe Asp Asp Val Ser Phe
20 25 30

Val His His Ala Leu Ala Gly Ile Asp Arg Pro Asp Val Arg Leu Ala
35 40 45

Thr Thr Phe Ala Gly Ile Thr Trp Arg Leu Pro Leu Tyr Ile Asn Ala
50 55 60

Met Thr Gly Gly Ser Ala Lys Thr Gly Ala Ile Asn Arg Asp Leu Ala
65 70 75 80

Val Ala Ala Arg Glu Thr Gly Ala Ala Ile Ala Ser Gly Ser Met His
85 90 95

Ala Phe Phe Arg Asp Pro Ser Cys Ala Asp Thr Phe Arg Val Leu Arg
100 105 110

Thr Glu Asn Pro Asp Gly Phe Val Met Ala Asn Val Asn Ala Thr Ala
115 120 125

Ser Val Asp Asn Ala Arg Arg Ala Val Asp Leu Ile Glu Ala Asn Ala
130 135 140

Leu Gln Ile His Leu Asn Thr Ala Gln Glu Thr Pro Met Pro Glu Gly
145 150 155 160

Asp Arg Ser Phe Gly Ser Trp Pro Ala Gln Ile Ala Lys Ile Thr Ala
165 170 175

Ala Val Asp Val Pro Val Ile Val Lys Glu Val Gly Asn Gly Leu Ser
180 185 190

Arg Gln Thr Leu Leu Ala Leu Pro Asp Leu Gly Val Arg Val Ala Asp

195 200 205

Val Ser Gly Arg Gly Gly Thr Asp Phe Ala Arg Ile Glu Asn Ser Arg
210 215 220

Arg Pro Leu Gly Asp Tyr Ala Phe Leu His Gly Trp Gly Gln Ser Thr
225 230 235 240

Pro Ala Cys Leu Leu Asp Ala Gln Asp Val Gly Phe Pro Leu Leu Ala
245 250 255

Ser Gly Gly Ile Arg Asn Pro Leu Asp Val Ala Arg Ala Leu Ala Leu
260 265 270

Gly Ala Gly Ala Val Gly Ser Ser Gly Val Phe Leu Arg Thr Leu Ile
275 280 285

Asp Gly Gly Val Ser Ala Leu Val Ala Gln Ile Ser Thr Trp Leu Asp
290 295 300

Gln Leu Ala Ala Leu Gln Thr Met Leu Gly Ala Arg Thr Pro Ala Asp
305 310 315 320

Leu Thr Arg Cys Asp Val Leu Ile His Gly Pro Leu Arg Ser Phe Cys
325 330 335

Thr Asp Arg Gly Ile Asp Ile Gly Arg Phe Ala Arg Arg Ser Ser Ser
340 345 350

Ala Asp Ile Arg Ser Glu Met Thr Gly Ser Thr Arg
355 360

<210> 62

<211> 368

<212> PRT

<213> Sulfolobus solfataricus

<400> 62

Met Pro Asp Ile Val Asn Arg Lys Val Glu His Val Glu Ile Ala Ala
1 5 10 15

Phe Glu Asn Val Asp Gly Leu Ser Ser Ser Thr Phe Leu Asn Asp Val
20 25 30

Ile Leu Val His Gln Gly Phe Pro Gly Ile Ser Phe Ser Glu Ile Asn
35 40 45

Thr Lys Thr Lys Phe Phe Arg Lys Glu Ile Ser Ala Pro Ile Met Val
50 55 60

Thr Gly Met Thr Gly Gly Arg Asn Glu Leu Gly Arg Ile Asn Arg Ile

65 70 75 80

Ile Ala Glu Val Ala Glu Lys Phe Gly Ile Pro Met Gly Val Gly Ser
85 90 95

Gln Arg Val Ala Ile Glu Lys Ala Glu Ala Arg Glu Ser Phe Thr Ile
100 105 110

Val Arg Lys Val Ala Pro Thr Ile Pro Ile Ile Ala Asn Leu Gly Met
115 120 125

Pro Gln Leu Val Lys Gly Tyr Gly Leu Lys Glu Phe Gln Asp Ala Ile
130 135 140

Gln Met Ile Glu Ala Asp Ala Ile Ala Val His Leu Asn Pro Ala Gln
145 150 155 160

Glu Val Phe Gln Pro Glu Gly Glu Pro Glu Tyr Gln Ile Tyr Ala Leu
165 170 175

Glu Arg Leu Arg Asp Ile Ser Lys Glu Leu Ser Val Pro Ile Ile Val
180 185 190

Lys Glu Ser Gly Asn Gly Ile Ser Met Glu Thr Ala Lys Leu Leu Tyr
195 200 205

Ser Tyr Gly Ile Lys Asn Phe Asp Thr Ser Gly Gln Gly Gly Thr Asn
210 215 220

Trp Ile Ala Ile Glu Met Ile Arg Asp Ile Arg Arg Gly Asn Trp Lys
225 230 235 240

Ala Glu Ser Ala Lys Asn Phe Leu Asp Trp Gly Val Pro Thr Ala Ala
245 250 255

Ser Ile Ile Glu Val Arg Tyr Ser Ile Pro Asp Ala Phe Leu Val Gly
260 265 270

Ser Gly Gly Ile Arg Ser Gly Leu Asp Ala Ala Lys Ala Ile Ala Leu
275 280 285

Gly Ala Asp Ile Ala Gly Met Ala Leu Pro Val Leu Lys Ser Ala Ile
290 295 300

Glu Gly Lys Glu Ser Leu Glu Gln Phe Phe Arg Lys Ile Ile Phe Glu
305 310 315 320

Leu Lys Ala Thr Met Met Leu Thr Gly Ser Lys Asn Val Glu Ala Leu
325 330 335

Lys Arg Ser Ser Ile Val Ile Leu Gly Lys Leu Lys Glu Trp Ala Glu
340 345 350

Tyr Arg Gly Ile Asn Leu Ser Ile Tyr Glu Lys Val Arg Lys Arg Glu
355 360 365

<210> 63

<211> 342

<212> PRT

<213> Rickettsia prowazekii

<400> 63

Met Pro Lys Glu Gln Asn Leu Asp Ile Glu Arg Lys Gln Glu His Ile
1 5 10 15

Glu Ile Asn Leu Lys Gln Asn Val Asn Ser Thr Leu Lys Ser Gly Leu
20 25 30

Glu Ser Ile Lys Phe Ile His Asn Ala Leu Pro Glu Ile Asn Tyr Asp
35 40 45

Ser Ile Asp Thr Thr Thr Thr Phe Leu Gly Lys Asp Met Lys Ala Pro
50 55 60

Ile Leu Ile Ser Ser Met Thr Gly Gly Thr Ala Arg Ala Arg Asp Ile
65 70 75 80

Asn Tyr Arg Leu Ala Gln Ala Ala Gln Lys Ser Gly Ile Ala Met Gly
85 90 95

Leu Gly Ser Met Arg Ile Leu Leu Thr Lys Pro Asp Thr Ile Lys Thr
100 105 110

Phe Thr Val Arg His Val Ala Pro Asp Ile Pro Leu Leu Ala Asn Ile
115 120 125

Gly Ala Val Gln Leu Asn Tyr Gly Val Thr Pro Lys Glu Cys Gln Tyr
130 135 140

Leu Ile Asp Thr Ile Lys Ala Asp Ala Leu Ile Leu His Leu Asn Val
145 150 155 160

Leu His Glu Leu Thr Gln Pro Glu Gly Asn Lys Asn Trp Glu Asn Leu
165 170 175

Leu Pro Lys Ile Lys Glu Val Ile Asn Tyr Leu Ser Val Pro Val Ile
180 185 190

Val Lys Glu Val Gly Tyr Gly Leu Ser Lys Gln Val Ala Lys Lys Leu
195 200 205

Ile Lys Ala Gly Val Lys Val Leu Asp Ile Ala Gly Ser Gly Gly Thr
210 215 220

Ser Trp Ser Gln Val Glu Ala Tyr Arg Ala Lys Asn Ser Met Gln Asn
225 230 235 240

Arg Ile Ala Ser Ser Phe Ile Asn Trp Gly Ile Thr Thr Leu Asp Ser

245 250 255

Leu Lys Met Leu Gln Glu Ile Ser Lys Asp Ile Thr Ile Ile Ala Ser
260 265 270

Gly Gly Leu Gln Ser Gly Ile Asp Gly Ala Lys Ala Ile Arg Met Gly
275 280 285

Ala Asn Ile Phe Gly Leu Ala Gly Lys Leu Leu Lys Ala Ala Asp Ile
290 295 300

Ala Glu Ser Leu Val Leu Glu Glu Ile Gln Val Ile Ile Glu Gln Leu
305 310 315 320

Lys Ile Thr Met Leu Cys Thr Gly Ser Cys Thr Leu Lys Asp Leu Ala
325 330 335

Lys Ala Glu Ile Met Trp
340

<210> 64

<211> 286

<212> PRT

<213> Deinococcus radiodurans

<400> 64

Met Arg Leu Asp Thr Val Phe Leu Gly Arg Arg Leu Lys Ala Pro Val
1 5 10 15

Leu Ile Gly Ala Met Thr Gly Gly Ala Glu Lys Ala Gly Val Ile Asn
20 25 30

Arg Asn Leu Ala Thr Ala Ala Arg Asn Leu Gly Leu Gly Met Met Leu
35 40 45

Gly Ser Gln Arg Val Met Leu Glu His Pro Asp Ala Trp Glu Ser Phe
50 55 60

Asn Val Arg Glu Val Ala Pro Glu Ile Leu Leu Ile Gly Asn Leu Gly
65 70 75 80

Ala Ala Gln Phe Met Leu Gly Tyr Gly Ala Glu Gln Ala Arg Arg Ala
85 90 95

Val Asp Glu Val Met Ala Asp Ala Leu Ala Ile His Leu Asn Pro Leu
100 105 110

Gln Glu Ala Leu Gln Arg Gly Gly Asp Thr Arg Trp Gln Gly Val Thr
115 120 125

Tyr Arg Leu Lys Gln Val Ala Arg Glu Leu Asp Phe Pro Val Ile Ile

130 135 140

Lys Glu Val Gly His Gly Leu Asp Ala Ala Thr Leu Arg Ala Leu Ala
145 150 155 160

Asp Gly Pro Phe Ala Ala Tyr Asp Val Ala Gly Ala Gly Gly Thr Ser
165 170 175

Trp Ala Arg Val Glu Gln Leu Val Ala His Gly Gln Val His Ser Pro
180 185 190

Asp Leu Cys Glu Leu Gly Val Pro Thr Ala Gln Ala Leu Arg Gln Ala
195 200 205

Arg Lys Thr Leu Pro Gly Ala Gln Leu Ile Ala Ser Gly Gly Ile Arg
210 215 220

Ser Gly Leu Asp Ala Ala Arg Ala Leu Ser Leu Gly Ala Glu Val Val
225 230 235 240

Ala Val Ala Arg Pro Leu Leu Glu Pro Ala Leu Asp Ser Ser Glu Ala
245 250 255

Ala Glu Ala Trp Leu Arg Asn Phe Ile Gln Glu Leu Arg Val Ala Leu
260 265 270

Phe Val Gly Gly Tyr Arg Asp Val Arg Glu Val Arg Gly Gly
275 280 285

<210> 65

<211> 361

<212> PRT

<213> Aeropyrum pernix

<400> 65

Met Ile Val Ser Ser Lys Val Glu Ser Arg Glu Ser Thr Leu Leu Glu
1 5 10 15

Tyr Val Arg Ile Val His Asn Pro Thr Pro Glu Val Asn Leu Gly Asp
20 25 30

Val Ser Leu Glu Ile Asp Phe Cys Gly Gly Arg Leu Arg Ala Pro Leu
35 40 45

Val Ile Thr Gly Met Thr Gly Gly His Pro Asp Val Glu Trp Ile Asn
50 55 60

Arg Glu Leu Ala Ser Val Ala Glu Glu Leu Gly Ile Ala Ile Gly Val
65 70 75 80

Gly Ser Gln Arg Ala Ala Ile Glu Asp Pro Ser Leu Ala Arg Thr Phe

85 90 95

Arg Ala Ala Arg Glu Ala Ala Pro Asn Ala Phe Leu Ile Ala Asn Leu
100 105 110

Gly Ala Pro Gln Leu Ser Leu Gly Tyr Ser Val Arg Glu Val Arg Met
115 120 125

Ala Val Glu Met Ile Asp Ala Asp Ala Ile Ala Ile His Leu Asn Pro
130 135 140

Gly Gln Glu Ala Tyr Gln Pro Glu Gly Asp Pro Phe Tyr Arg Gly Val
145 150 155 160

Val Gly Lys Ile Ala Glu Ala Ala Glu Ala Ala Gly Val Pro Val Ile
165 170 175

Val Lys Glu Thr Gly Asn Gly Leu Ser Arg Glu Ala Val Ala Gln Leu
180 185 190

Arg Ala Leu Gly Val Arg Cys Phe Asp Val Ala Gly Leu Gly Gly Thr
195 200 205

Asn Trp Ile Lys Ile Glu Val Leu Arg Gly Arg Lys Ala Gly Ser Pro
210 215 220

Leu Glu Ala Gly Pro Leu Gln Asp Phe Trp Gly Asn Pro Thr Ala Ala
225 230 235 240

Ala Leu Met Glu Ala Arg Thr Ala Ala Pro Asp Ala Tyr Ile Ile Ala
245 250 255

Ser Gly Gly Val Arg Asn Gly Leu Asp Ala Ala Arg Ala Ile Ala Leu
260 265 270

Gly Ala Asp Ala Ala Gly Val Ala Leu Pro Ala Ile Arg Ser Leu Leu
275 280 285

Ser Gly Gly Arg Gln Ala Thr Leu Lys Leu Leu Lys Ala Ile Glu Tyr
290 295 300

Gln Leu Lys Thr Ala Val Tyr Met Val Gly Glu Thr Arg Val Arg Gly
305 310 315 320

Leu Trp Arg Ala Pro Ile Val Val Trp Gly Arg Leu Ala Glu Glu Ala
325 330 335

Glu Ala Arg Gly Ile Asp Pro Arg Trp Tyr Thr Asn Thr Leu Arg Leu
340 345 350

Glu Ala Leu Val Tyr Lys Asp Val Lys
355 360

<210> 66

<211> 379

<212> PRT

<213> Halobacterium sp. NRC-1

<400> 66

Met Gly Glu Ser Arg Tyr Asn Ser Ile Val Phe Pro Ser Leu Val Gln
1 5 10 15

Thr Arg Leu Met Thr Ala Gln Asp Ser Thr Gln Thr Glu Asp Arg Lys
20 25 30

Asp Asp His Leu Gln Ile Val Gln Glu Arg Asp Val Glu Thr Thr Gly
35 40 45

Thr Gly Phe Asp Asp Val His Leu Val His Asn Ala Leu Pro Glu Leu
50 55 60

Asp Tyr Asp Ala Ile Asp Pro Ser Ile Asp Phe Leu Gly His Asp Leu
65 70 75 80

Ser Ala Pro Ile Phe Ile Glu Ser Met Thr Gly Gly His His Asn Thr
85 90 95

Thr Glu Ile Asn Arg Ala Leu Ala Arg Ala Ala Ser Glu Thr Gly Ile
100 105 110

Ala Met Gly Leu Gly Ser Gln Arg Ala Gly Leu Glu Leu Asp Asp Glu
115 120 125

Arg Val Leu Glu Ser Tyr Thr Val Val Arg Asp Ala Ala Pro Asp Ala
130 135 140

Phe Ile Tyr Gly Asn Leu Gly Ala Ala Gln Leu Arg Glu Tyr Asp Ile
145 150 155 160

Glu Met Val Glu Gln Ala Val Glu Met Ile Asp Ala Asp Ala Leu Ala
165 170 175

Val His Leu Asn Phe Leu Gln Glu Ala Thr Gln Pro Glu Gly Asp Val
180 185 190

Asp Gly Arg Asn Cys Val Ala Ala Ile Glu Arg Val Ser Glu Ala Leu
195 200 205

Ser Val Pro Ile Ile Val Lys Glu Thr Gly Asn Gly Ile Ser Gly Glu
210 215 220

Thr Ala Arg Glu Leu Thr Ala Ala Gly Val Asp Ala Leu Asp Val Ala
225 230 235 240

Gly Lys Gly Gly Thr Thr Trp Ser Gly Ile Glu Ala Tyr Arg Ala Ala
245 250 255

Ala Ala Asn Ala Pro Arg Gln Lys Gln Ile Gly Thr Leu Phe Arg Glu

260 265 270

Trp Gly Ile Pro Thr Ala Ala Ser Thr Ile Glu Cys Val Ala Glu His
275 280 285

Asp Cys Val Ile Ala Ser Gly Gly Val Arg Thr Gly Leu Asp Val Ala
290 295 300

Lys Ala Ile Ala Leu Gly Ala Arg Ala Gly Gly Leu Ala Lys Pro Phe
305 310 315 320

Leu Lys Pro Ala Thr Asp Gly Pro Asp Ala Val Ile Glu Arg Val Gly
325 330 335

Asp Leu Ile Ala Glu Leu Arg Thr Ala Met Phe Val Thr Gly Ser Gly
340 345 350

Ser Ile Asp Glu Leu Gln Gln Val Glu Tyr Val Leu His Gly Lys Thr
355 360 365

Arg Glu Tyr Val Glu Gln Arg Thr Ser Ser Glu
370 375

<210> 67

<211> 317

<212> PRT

<213> Archaeoglobus fulgidus

<400> 67

Met Met Leu Ile His Lys Ala Leu Pro Glu Val Asp Tyr Trp Lys Ile
1 5 10 15

Asp Thr Glu Ile Glu Phe Phe Gly Lys Lys Leu Ser Phe Pro Leu Leu
20 25 30

Ile Ala Ser Met Thr Gly Gly His Pro Glu Thr Lys Glu Ile Asn Ala
35 40 45

Arg Leu Gly Glu Ala Val Glu Glu Ala Gly Ile Gly Met Gly Val Gly
50 55 60

Ser Gln Arg Ala Ala Ile Glu Asp Glu Ser Leu Ala Asp Ser Phe Thr
65 70 75 80

Val Val Arg Glu Lys Ala Pro Asn Ala Phe Val Tyr Ala Asn Ile Gly
85 90 95

Met Pro Gln Val Ile Glu Arg Gly Val Glu Ile Val Asp Arg Ala Val
100 105 110

Glu Met Ile Asp Ala Asp Ala Val Ala Ile His Leu Asn Tyr Leu Gln

115 120 125

Glu Ala Ile Gln Pro Glu Gly Asp Leu Asn Ala Glu Lys Gly Leu Glu
130 135 140

Val Leu Glu Glu Val Cys Arg Ser Val Lys Val Pro Val Ile Ala Lys
145 150 155 160

Glu Thr Gly Ala Gly Ile Ser Arg Glu Val Ala Val Met Leu Lys Arg
165 170 175

Ala Gly Val Ser Ala Ile Asp Val Gly Gly Lys Gly Gly Thr Thr Phe
180 185 190

Ser Gly Val Glu Val Tyr Arg Val Asn Asp Glu Val Ser Lys Ser Val
195 200 205

Gly Ile Asp Phe Trp Asp Trp Gly Leu Pro Thr Ala Phe Ser Ile Val
210 215 220

Asp Cys Arg Gly Ile Leu Pro Val Ile Ala Thr Gly Gly Leu Arg Ser
225 230 235 240

Gly Leu Asp Val Ala Lys Ser Ile Ala Ile Gly Ala Glu Leu Gly Ser
245 250 255

Ala Ala Leu Pro Phe Leu Arg Ala Ala Val Glu Ser Ala Glu Lys Val
260 265 270

Arg Glu Glu Ile Glu Tyr Phe Arg Arg Gly Leu Lys Thr Ala Met Phe
275 280 285

Leu Thr Gly Cys Lys Asn Val Glu Glu Leu Lys Gly Leu Lys Val Phe
290 295 300

Val Ser Gly Arg Leu Lys Glu Trp Ile Asp Phe Arg Gly
305 310 315

<210> 68

<211> 370

<212> PRT

<213> Pyrococcus abyssi

<400> 68

Met Glu Glu Gln Thr Ile Leu Arg Lys Phe Glu His Ile Lys His Cys
1 5 10 15

Leu Thr Lys Asn Val Glu Ala His Val Thr Asn Gly Phe Glu Asp Val
20 25 30

His Leu Ile His Lys Ser Leu Pro Glu Ile Asp Lys Asp Glu Ile Asp

35 40 45

Leu Ser Val Lys Phe Leu Gly Arg Lys Phe Asp Tyr Pro Ile Met Ile
50 55 60

Thr Gly Met Thr Gly Gly Thr Arg Lys Gly Glu Ile Ala Trp Arg Ile
65 70 75 80

Asn Arg Thr Leu Ala Gln Ala Ala Gln Glu Leu Asn Ile Pro Leu Gly
85 90 95

Leu Gly Ser Gln Arg Ala Met Ile Glu Lys Pro Glu Thr Trp Glu Ser
100 105 110

Tyr Tyr Val Arg Asp Val Ala Pro Asp Val Phe Leu Val Gly Asn Leu
115 120 125

Gly Ala Pro Gln Phe Gly Arg Asn Ala Lys Lys Arg Tyr Ser Val Asp
130 135 140

Glu Val Leu Tyr Ala Ile Glu Lys Ile Glu Ala Asp Ala Ile Ala Ile
145 150 155 160

His Met Asn Pro Leu Gln Glu Ser Ile Gln Pro Glu Gly Asp Thr Thr
165 170 175

Phe Ser Gly Val Leu Glu Ala Leu Ala Glu Ile Thr Ser Thr Ile Asp
180 185 190

Tyr Pro Val Ile Ala Lys Glu Thr Gly Ala Gly Val Ser Lys Glu Val
195 200 205

Ala Val Glu Leu Glu Ala Val Gly Val Asp Ala Ile Asp Ile Ser Gly
210 215 220

Leu Gly Gly Thr Ser Trp Ser Ala Val Glu Tyr Tyr Arg Thr Lys Asp
225 230 235 240

Gly Glu Lys Arg Asn Leu Ala Leu Lys Phe Trp Asp Trp Gly Ile Lys
245 250 255

Thr Ala Ile Ser Leu Ala Glu Val Arg Trp Ala Thr Asn Leu Pro Ile
260 265 270

Ile Ala Ser Gly Gly Met Arg Asp Gly Ile Thr Met Ala Lys Ala Leu
275 280 285

Ala Met Gly Ala Ser Met Val Gly Ile Ala Leu Pro Val Leu Arg Pro
290 295 300

Ala Ala Lys Gly Asp Val Glu Gly Val Ile Arg Ile Ile Lys Gly Tyr
305 310 315 320

Ala Glu Glu Ile Arg Asn Val Met Phe Leu Val Gly Ala Arg Asn Ile
325 330 335

Lys Glu Leu Arg Lys Val Pro Leu Val Ile Thr Gly Phe Val Arg Glu

340 345 350

Trp Leu Leu Gln Arg Ile Asp Leu Asn Ser Tyr Leu Arg Ala Arg Phe
355 360 365

Lys Met
370

<210> 69

<211> 371

<212> PRT

<213> Pyrococcus horikoshii

<400> 69

Met Lys Glu Glu Leu Thr Ile Leu Arg Lys Phe Glu His Ile Glu His
1 5 10 15

Cys Leu Lys Arg Asn Val Glu Ala His Val Ser Asn Gly Phe Glu Asp
20 25 30

Val Tyr Phe Val His Lys Ser Leu Pro Glu Ile Asp Lys Asp Glu Ile
35 40 45

Asp Leu Thr Val Glu Phe Leu Gly Arg Lys Phe Asp Tyr Pro Ile Met
50 55 60

Ile Thr Gly Met Thr Gly Gly Thr Arg Arg Glu Glu Ile Ala Gly Lys
65 70 75 80

Ile Asn Arg Thr Leu Ala Met Ala Ala Glu Glu Leu Asn Ile Pro Phe
85 90 95

Gly Val Gly Ser Gln Arg Ala Met Ile Glu Lys Pro Glu Thr Trp Glu
100 105 110

Ser Tyr Tyr Val Arg Asp Val Ala Pro Asp Ile Phe Leu Ile Gly Asn
115 120 125

Leu Gly Ala Pro Gln Phe Gly Lys Asn Ala Lys Lys Arg Tyr Ser Val
130 135 140

Lys Glu Val Leu Tyr Ala Ile Glu Lys Ile Glu Ala Asp Ala Ile Ala
145 150 155 160

Ile His Met Asn Pro Leu Gln Glu Ser Val Gln Pro Glu Gly Asp Thr
165 170 175

Thr Tyr Ala Gly Val Leu Glu Ala Leu Ala Glu Ile Lys Ser Ser Ile
180 185 190

Asn Tyr Pro Val Ile Ala Lys Glu Thr Gly Ala Gly Val Ser Lys Glu

195 200 205

Val Ala Ile Glu Leu Glu Ser Val Gly Ile Asp Ala Ile Asp Ile Ser
210 215 220

Gly Leu Gly Gly Thr Ser Trp Ser Ala Val Glu Tyr Tyr Arg Ala Lys
225 230 235 240

Asp Ser Glu Lys Arg Lys Ile Ala Leu Lys Phe Trp Asp Trp Gly Ile
245 250 255

Lys Thr Ala Ile Ser Leu Ala Glu Val Arg Trp Ala Thr Asn Leu Pro
260 265 270

Ile Ile Ala Ser Gly Gly Met Arg Asp Gly Val Met Met Ala Lys Ala
275 280 285

Leu Ala Met Gly Ala Ser Leu Val Gly Ile Ala Leu Pro Val Leu Arg
290 295 300

Pro Ala Ala Arg Gly Asp Val Glu Gly Val Val Arg Ile Ile Arg Gly
305 310 315 320

Tyr Ala Glu Glu Ile Lys Asn Val Met Phe Leu Val Gly Ala Arg Asn
325 330 335

Ile Arg Glu Leu Arg Arg Val Pro Leu Val Ile Thr Gly Phe Val Arg
340 345 350

Glu Trp Leu Leu Gln Arg Ile Asp Leu Asn Ser Tyr Leu Arg Ser Arg
355 360 365

Phe Lys His
370

<210> 70

<211> 349

<212> PRT

<213> Methanobacterium thermoautotrophicum

<400> 70

Met Ile Ser Asp Arg Lys Leu Glu His Leu Ile Leu Cys Ala Ser Cys
1 5 10 15

Asp Val Glu Tyr Arg Lys Lys Thr Gly Phe Glu Asp Ile Glu Ile Val
20 25 30

His Arg Ala Ile Pro Glu Ile Asn Lys Glu Lys Ile Asp Ile Ser Leu
35 40 45

Asp Phe Leu Gly Arg Glu Leu Ser Ser Pro Val Met Ile Ser Ala Ile

50 55 60

Thr Gly Gly His Pro Ala Ser Met Lys Ile Asn Arg Glu Leu Ala Arg
65 70 75 80

Ala Ala Glu Lys Leu Gly Ile Ala Leu Gly Leu Gly Ser Gln Arg Ala
85 90 95

Gly Val Glu His Pro Glu Leu Glu Gly Thr Tyr Thr Ile Ala Arg Glu
100 105 110

Glu Ala Pro Ser Ala Met Leu Ile Gly Asn Ile Gly Ser Ser His Ile
115 120 125

Glu Tyr Ala Glu Arg Ala Val Glu Met Ile Asp Ala Asp Ala Leu Ala
130 135 140

Val His Leu Asn Pro Leu Gln Glu Ser Ile Gln Pro Gly Gly Asp Val
145 150 155 160

Asp Ser Ser Gly Ala Leu Glu Ser Ile Ser Ala Ile Val Glu Ser Val
165 170 175

Asp Val Pro Val Met Val Lys Glu Thr Gly Ala Gly Ile Cys Ser Glu
180 185 190

Asp Ala Ile Glu Leu Glu Ser Cys Gly Val Ser Ala Ile Asp Val Ala
195 200 205

Gly Ala Gly Gly Thr Ser Trp Ala Ala Val Glu Thr Tyr Arg Ala Asp
210 215 220

Asp Arg Tyr Leu Gly Glu Leu Phe Trp Asp Trp Gly Ile Pro Thr Ala
225 230 235 240

Ala Ser Thr Val Glu Val Val Glu Ser Val Ser Ile Pro Val Ile Ala
245 250 255

Ser Gly Gly Ile Arg Ser Gly Ile Asp Ala Ala Lys Ala Ile Ser Leu
260 265 270

Gly Ala Glu Met Val Gly Ile Ala Leu Pro Val Leu Glu Ala Ala Gly
275 280 285

His Gly Tyr Arg Glu Val Ile Lys Val Ile Glu Gly Phe Asn Glu Ala
290 295 300

Leu Arg Thr Ala Met Tyr Leu Ala Gly Ala Glu Thr Leu Asp Asp Leu
305 310 315 320

Lys Lys Ser Pro Val Ile Ile Thr Gly His Thr Gly Glu Trp Leu Asn
325 330 335

Gln Arg Gly Phe Glu Thr Lys Lys Tyr Ala Arg Arg Ser
340 345

<210> 71

<211> 359

<212> PRT

<213> Methanococcus jannaschii

<400> 71

Met Val Asn Asn Arg Asn Glu Ile Glu Val Arg Lys Leu Glu His Ile
1 5 10 15

Phe Leu Cys Ser Tyr Cys Asn Val Glu Tyr Glu Lys Thr Thr Leu Leu
20 25 30

Glu Asp Ile Glu Leu Ile His Lys Gly Thr Cys Gly Ile Asn Phe Asn
35 40 45

Asp Ile Glu Thr Glu Ile Glu Leu Phe Gly Lys Lys Leu Ser Ala Pro
50 55 60

Ile Ile Val Ser Gly Met Thr Gly Gly His Ser Lys Ala Lys Glu Ile
65 70 75 80

Asn Lys Asn Ile Ala Lys Ala Val Glu Glu Leu Gly Leu Gly Met Gly
85 90 95

Val Gly Ser Gln Arg Ala Ala Ile Val Asn Asp Glu Leu Ile Asp Thr
100 105 110

Tyr Ser Ile Val Arg Asp Tyr Thr Asn Asn Leu Val Ile Gly Asn Leu
115 120 125

Gly Ala Val Asn Phe Ile Val Asp Asp Trp Asp Glu Glu Ile Ile Asp
130 135 140

Lys Ala Ile Glu Met Ile Asp Ala Asp Ala Ile Ala Ile His Phe Asn
145 150 155 160

Pro Leu Gln Glu Ile Ile Gln Pro Glu Gly Asp Leu Asn Phe Lys Asn
165 170 175

Leu Tyr Lys Leu Lys Glu Ile Ile Ser Asn Tyr Lys Lys Ser Tyr Lys
180 185 190

Asn Ile Pro Phe Ile Ala Lys Gln Val Gly Glu Gly Phe Ser Lys Glu
195 200 205

Asp Ala Leu Ile Leu Lys Asp Ile Gly Phe Asp Ala Ile Asp Val Gln
210 215 220

Gly Ser Gly Gly Thr Ser Trp Ala Lys Val Glu Ile Tyr Arg Val Lys
225 230 235 240

Glu Glu Glu Ile Lys Arg Leu Ala Glu Lys Phe Ala Asn Trp Gly Ile

245 250 255

Pro Thr Ala Ala Ser Ile Phe Glu Val Lys Ser Val Tyr Asp Gly Ile
260 265 270

Val Ile Gly Ser Gly Gly Ile Arg Gly Gly Leu Asp Ile Ala Lys Cys
275 280 285

Ile Ala Ile Gly Cys Asp Cys Cys Ser Val Ala Leu Pro Ile Leu Lys
290 295 300

Ala Ser Leu Lys Gly Trp Glu Glu Val Val Lys Val Leu Glu Ser Tyr
305 310 315 320

Ile Lys Glu Leu Lys Ile Ala Met Phe Leu Val Gly Ala Glu Asn Ile
325 330 335

Glu Glu Leu Lys Lys Thr Ser Tyr Ile Val Lys Gly Thr Leu Lys Glu
340 345 350

Trp Ile Ser Gln Arg Leu Lys
355

<210> 72

<211> 348

<212> PRT

<213> Thermoplasma acidophilum

<400> 72

Met Ile Gly Lys Arg Lys Glu Glu His Ile Arg Ile Ala Glu Asn Glu
1 5 10 15

Asp Val Ser Ser Phe His Asn Phe Trp Asp Asp Ile Ser Leu Met His
20 25 30

Glu Ala Asp Pro Glu Val Asn Tyr Asp Glu Ile Asp Thr Ser Val Asp
35 40 45

Phe Leu Gly Lys Lys Leu Lys Phe Pro Met Ile Ile Ser Ser Met Thr
50 55 60

Gly Gly Ala Glu Ile Ala Lys Asn Ile Asn Arg Asn Leu Ala Val Ala
65 70 75 80

Ala Glu Arg Phe Gly Ile Gly Met Gly Val Gly Ser Met Arg Ala Ala
85 90 95

Ile Val Asp Arg Ser Ile Glu Asp Thr Tyr Ser Val Ile Asn Glu Ser
100 105 110

His Val Pro Leu Lys Ile Ala Asn Ile Gly Ala Pro Gln Leu Val Arg

115 120 125

Gln Asp Lys Asp Ala Val Ser Asn Arg Asp Ile Ala Tyr Ile Tyr Asp
130 135 140

Leu Ile Lys Ala Asp Phe Leu Ala Val His Phe Asn Phe Leu Gln Glu
145 150 155 160

Met Val Gln Pro Glu Gly Asp Arg Asn Ser Lys Gly Val Ile Asp Arg
165 170 175

Ile Lys Asp Leu Ser Gly Ser Phe Asn Ile Ile Ala Lys Glu Thr Gly
180 185 190

Ser Gly Phe Ser Arg Arg Thr Ala Glu Arg Leu Ile Asp Ala Gly Val
195 200 205

Lys Ala Ile Glu Val Ser Gly Val Ser Gly Thr Thr Phe Ala Ala Val
210 215 220

Glu Tyr Tyr Arg Ala Arg Lys Glu Asn Asn Leu Glu Lys Met Arg Ile
225 230 235 240

Gly Glu Thr Phe Trp Asn Trp Gly Ile Pro Ser Pro Ala Ser Val Tyr
245 250 255

Tyr Cys Ser Asp Leu Ala Pro Val Ile Gly Ser Gly Gly Leu Arg Asn
260 265 270

Gly Leu Asp Leu Ala Lys Ala Ile Ala Met Gly Ala Thr Ala Gly Gly
275 280 285

Phe Ala Arg Ser Leu Leu Lys Asp Ala Asp Thr Asp Pro Glu Met Leu
290 295 300

Met Lys Asn Ile Glu Leu Ile Gln Arg Glu Phe Arg Val Ala Leu Phe
305 310 315 320

Leu Thr Gly Asn Lys Asn Val Tyr Glu Leu Lys Phe Thr Lys Lys Val
325 330 335

Ile Val Asp Pro Leu Arg Ser Trp Leu Glu Ala Lys
340 345

<210> 73

<211> 357

<212> PRT

<213> Leishmania major

<400> 73

Met Ser Ser Arg Asp Cys Thr Val Asp Arg Glu Ala Ala Val Gln Lys

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1               5                   10                  15
Arg Lys Lys Asp His Ile Asp Ile Cys Leu His Gln Asp Val Glu Pro
            20                  25                  30
His Lys Arg Arg Thr Ser Ile Trp Asn Lys Tyr Thr Leu Pro Tyr Lys
        35                  40                  45
Ala Leu Pro Glu Val Asp Leu Gln Lys Ile Asp Thr Ser Cys Glu Phe
    50                  55                  60
Met Gly Lys Arg Ile Ser Phe Pro Phe Phe Ile Ser Ser Met Thr Gly
65                  70                  75                  80
Gly Glu Ala His Gly Arg Val Ile Asn Glu Asn Leu Ala Lys Ala Cys
                85                  90                  95
Glu Ala Glu Lys Ile Pro Phe Gly Leu Gly Ser Met Arg Ile Ile Asn
            100                 105                 110
Arg Tyr Ala Ser Ala Val His Thr Phe Asn Val Lys Glu Phe Cys Pro
        115                 120                 125
Ser Val Pro Met Leu Ala Asn Ile Gly Leu Val Gln Leu Asn Tyr Gly
    130                 135                 140
Phe Gly Pro Lys Glu Val Asn Asn Leu Val Asn Ser Val Arg Ala Asp
145                 150                 155                 160
Gly Leu Cys Ile His Leu Asn His Thr Gln Glu Val Cys Gln Pro Glu
                165                 170                 175
Gly Asp Thr Asn Phe Glu Gly Leu Ile Glu Lys Leu Arg Gln Leu Leu
            180                 185                 190
Pro His Ile Lys Val Pro Val Leu Val Lys Gly Val Gly His Gly Ile
        195                 200                 205
Asp Tyr Glu Ser Met Val Ala Ile Lys Ala Ser Gly Val Lys Tyr Val
    210                 215                 220
Asp Val Ser Gly Cys Gly Gly Thr Ser Trp Ala Trp Ile Glu Gly Arg
225                 230                 235                 240
Arg Gln Pro Tyr Lys Ala Glu Glu Glu Asn Ile Gly Tyr Leu Leu Arg
                245                 250                 255
Asp Ile Gly Val Pro Thr Asp Val Cys Leu Arg Glu Ser Ala Pro Leu
            260                 265                 270
Thr Val Asn Gly Asp Leu His Leu Ile Ala Gly Gly Gly Ile Arg Asn
        275                 280                 285
Gly Met Asp Val Ala Lys Ala Leu Met Met Gly Ala Glu Tyr Ala Thr
    290                 295                 300
Ala Ala Met Pro Phe Leu Ala Ala Ala Leu Glu Ser Ser Glu Ala Val
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305 310 315 320

Arg Ala Val Ile Gln Arg Met Arg Gln Glu Leu Arg Val Ser Met Phe
325 330 335

Thr Cys Gly Ala Arg Asn Ile Glu Glu Leu Arg Arg Met Lys Val Ile
340 345 350

Glu Leu Gly His Leu
355

<210> 74

<211> 398

<212> PRT

<213> Streptococcus pneumoniae

<400> 74

Met Asn Asp Lys Thr Glu Val Asn Met Thr Ile Gly Ile Asp Lys Ile
1 5 10 15

Gly Phe Ala Thr Ser Gln Tyr Val Leu Lys Leu Gln Asp Leu Ala Glu
20 25 30

Ala Arg Gly Ile Asp Pro Glu Lys Leu Ser Lys Gly Leu Leu Leu Lys
35 40 45

Glu Leu Ser Ile Ala Pro Leu Thr Glu Asp Ile Val Thr Leu Ala Ala
50 55 60

Ser Ala Ser Asp Ser Ile Leu Thr Glu Gln Glu Arg Gln Glu Val Asp
65 70 75 80

Met Val Ile Val Ala Thr Glu Ser Gly Ile Asp Gln Ser Lys Ala Ala
85 90 95

Ala Val Phe Val His Gly Leu Leu Gly Ile Gln Pro Phe Ala Arg Ser
100 105 110

Phe Glu Ile Lys Glu Ala Cys Tyr Gly Ala Thr Ala Ala Leu His Tyr
115 120 125

Ala Lys Leu His Val Glu Asn Ser Pro Glu Ser Lys Val Leu Val Ile
130 135 140

Ala Ser Asp Ile Ala Lys Tyr Gly Ile Glu Thr Pro Gly Glu Pro Thr
145 150 155 160

Gln Gly Ala Gly Ser Val Ala Met Leu Ile Thr Gln Asn Pro Arg Met
165 170 175

Met Ala Phe Asn Asn Asp Asn Val Ala Gln Thr Arg Asp Ile Met Asp

180 185 190

Phe Trp Arg Pro Asn Tyr Ser Thr Thr Pro Tyr Val Asn Gly Val Tyr
195 200 205

Ser Thr Gln Gln Tyr Leu Asp Ser Leu Lys Thr Thr Trp Leu Glu Tyr
210 215 220

Gln Lys Arg Tyr Gln Leu Thr Leu Asp Asp Phe Ala Ala Val Cys Phe
225 230 235 240

His Leu Pro Tyr Pro Lys Leu Ala Leu Lys Gly Leu Lys Lys Ile Met
245 250 255

Asp Lys Asn Leu Pro Gln Glu Lys Lys Asp Leu Leu Gln Lys His Phe
260 265 270

Asp Gln Ser Ile Leu Tyr Ser Gln Lys Val Gly Asn Ile Tyr Thr Gly
275 280 285

Ser Leu Phe Leu Gly Leu Leu Ser Leu Leu Glu Asn Thr Asp Ser Leu
290 295 300

Lys Ala Gly Asp Lys Ile Ala Leu Tyr Ser Tyr Gly Ser Gly Ala Val
305 310 315 320

Ala Glu Phe Phe Ser Gly Glu Leu Val Glu Gly Tyr Glu Ala Tyr Leu
325 330 335

Asp Lys Asp Arg Leu Asn Lys Leu Asn Gln Arg Thr Ala Leu Ser Val
340 345 350

Ala Asp Tyr Glu Lys Val Phe Phe Glu Glu Val Asn Leu Asp Glu Thr
355 360 365

Asn Ser Ala Gln Phe Ala Gly Tyr Glu Asn Gln Asp Phe Ala Leu Val
370 375 380

Glu Ile Leu Asp His Gln Arg Arg Tyr Ser Lys Val Glu Lys
385 390 395

<210> 75

<211> 391

<212> PRT

<213> Streptococcus pyrogenes

<400> 75

Met Thr Ile Gly Ile Asp Lys Ile Gly Phe Ala Thr Ser Gln Tyr Val
1 5 10 15

Leu Lys Leu Glu Asp Leu Ala Leu Ala Arg Gln Val Asp Pro Ala Lys

20 25 30

Phe Ser Gln Gly Leu Leu Ile Glu Ser Phe Ser Val Ala Pro Ile Thr
35 40 45

Glu Asp Ile Ile Thr Leu Ala Ala Ser Ala Ala Asp Gln Ile Leu Thr
50 55 60

Asp Glu Asp Arg Ala Lys Ile Asp Met Val Ile Leu Ala Thr Glu Ser
65 70 75 80

Ser Thr Asp Gln Ser Lys Ala Ser Ala Ile Tyr Val His His Leu Val
85 90 95

Gly Ile Gln Pro Phe Ala Arg Ser Phe Glu Val Lys Gln Ala Cys Tyr
100 105 110

Ser Ala Thr Ala Ala Leu Asp Tyr Ala Lys Leu His Val Ala Ser Lys
115 120 125

Pro Asp Ser Arg Val Leu Val Ile Ala Ser Asp Ile Ala Arg Tyr Gly
130 135 140

Val Gly Ser Pro Gly Glu Ser Thr Gln Gly Ser Gly Ser Ile Ala Leu
145 150 155 160

Leu Val Thr Ala Asp Pro Arg Ile Leu Ala Leu Asn Glu Asp Asn Val
165 170 175

Ala Gln Thr Arg Asp Ile Met Asp Phe Trp Arg Pro Asn Tyr Ser Phe
180 185 190

Thr Pro Tyr Val Asp Gly Ile Tyr Ser Thr Lys Gln Tyr Leu Asn Cys
195 200 205

Leu Glu Thr Thr Trp Gln Ala Tyr Gln Lys Arg Glu Asn Leu Gln Leu
210 215 220

Ser Asp Phe Ala Ala Val Cys Phe His Ile Pro Phe Pro Lys Leu Ala
225 230 235 240

Leu Lys Gly Leu Asn Asn Ile Met Asp Asn Thr Val Pro Pro Glu His
245 250 255

Arg Glu Lys Leu Ile Glu Ala Phe Gln Ala Ser Ile Thr Tyr Ser Lys
260 265 270

Gln Ile Gly Asn Ile Tyr Thr Gly Ser Leu Tyr Leu Gly Leu Leu Ser
275 280 285

Leu Leu Glu Asn Ser Lys Val Leu Gln Ser Gly Asp Lys Ile Gly Phe
290 295 300

Phe Ser Tyr Gly Ser Gly Ala Val Ser Glu Phe Tyr Ser Gly Gln Leu
305 310 315 320

Val Ala Gly Tyr Asp Lys Met Leu Met Thr Asn Arg Gln Ala Leu Leu

325 330 335

Asp Gln Arg Thr Arg Leu Ser Val Ser Lys Tyr Glu Asp Leu Phe Tyr
340 345 350

Glu Gln Val Gln Leu Asp Asp Asn Gly Asn Ala Asn Phe Asp Ile Tyr
355 360 365

Leu Thr Gly Lys Phe Ala Leu Thr Ala Ile Lys Glu His Gln Arg Ile
370 375 380

Tyr His Thr Asn Asp Lys Asn
385 390

<210> 76

<211> 383

<212> PRT

<213> Enterococcus faecalis

<400> 76

Met Thr Ile Gly Ile Asp Lys Ile Ser Phe Phe Val Pro Pro Tyr Tyr
1 5 10 15

Ile Asp Met Thr Ala Leu Ala Glu Ala Arg Asn Val Asp Pro Gly Lys
20 25 30

Phe His Ile Gly Ile Gly Gln Asp Gln Met Ala Val Asn Pro Ile Ser
35 40 45

Gln Asp Ile Val Thr Phe Ala Ala Asn Ala Ala Glu Ala Ile Leu Thr
50 55 60

Lys Glu Asp Lys Glu Ala Ile Asp Met Val Ile Val Gly Thr Glu Ser
65 70 75 80

Ser Ile Asp Glu Ser Lys Ala Ala Ala Val Val Leu His Arg Leu Met
85 90 95

Gly Ile Gln Pro Phe Ala Arg Ser Phe Glu Ile Lys Glu Ala Cys Tyr
100 105 110

Gly Ala Thr Ala Gly Leu Gln Leu Ala Lys Asn His Val Ala Leu His
115 120 125

Pro Asp Lys Lys Val Leu Val Val Ala Ala Asp Ile Ala Lys Tyr Gly
130 135 140

Leu Asn Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Leu Val Ala Ser Glu Pro Arg Ile Leu Ala Leu Lys Glu Asp Asn Val

165 170 175

Met Leu Thr Gln Asp Ile Tyr Asp Phe Trp Arg Pro Thr Gly His Pro
180 185 190

Tyr Pro Met Val Asp Gly Pro Leu Ser Asn Glu Thr Tyr Ile Gln Ser
195 200 205

Phe Ala Gln Val Trp Asp Glu His Lys Lys Arg Thr Gly Leu Asp Phe
210 215 220

Ala Asp Tyr Asp Ala Leu Ala Phe His Ile Pro Tyr Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Leu Ala Lys Ile Ser Asp Gln Thr Glu Ala Glu Gln
245 250 255

Glu Arg Ile Leu Ala Arg Tyr Glu Glu Ser Ile Ile Tyr Ser Arg Arg
260 265 270

Val Gly Asn Leu Tyr Thr Gly Ser Leu Tyr Leu Gly Leu Ile Ser Leu
275 280 285

Leu Glu Asn Ala Thr Thr Leu Thr Ala Gly Asn Gln Ile Gly Leu Phe
290 295 300

Ser Tyr Gly Ser Gly Ala Val Ala Glu Phe Phe Thr Gly Glu Leu Val
305 310 315 320

Ala Gly Tyr Gln Asn His Leu Gln Lys Glu Thr His Leu Ala Leu Leu
325 330 335

Asp Asn Arg Thr Glu Leu Ser Ile Ala Glu Tyr Glu Ala Met Phe Ala
340 345 350

Glu Thr Leu Asp Thr Asp Ile Asp Gln Thr Leu Glu Asp Glu Leu Lys
355 360 365

Tyr Ser Ile Ser Ala Ile Asn Asn Thr Val Arg Ser Tyr Arg Asn
370 375 380

<210> 77

<211> 384

<212> PRT

<213> Enterococcus faecium

<400> 77

Met Lys Ile Gly Ile Asp Arg Leu Ser Phe Phe Ile Pro Asn Leu Tyr
1 5 10 15

Leu Asp Met Thr Glu Leu Ala Glu Ser Arg Gly Asp Asp Pro Ala Lys

20 25 30

Tyr His Ile Gly Ile Gly Gln Asp Gln Met Ala Val Asn Arg Ala Asn
35 40 45

Glu Asp Ile Ile Thr Leu Gly Ala Asn Ala Ala Ser Lys Ile Val Thr
50 55 60

Glu Lys Asp Arg Glu Leu Ile Asp Met Val Ile Val Gly Thr Glu Ser
65 70 75 80

Gly Ile Asp His Ser Lys Ala Ser Ala Val Ile Ile His His Leu Leu
85 90 95

Lys Ile Gln Ser Phe Ala Arg Ser Phe Glu Val Lys Glu Ala Cys Tyr
100 105 110

Gly Gly Thr Ala Ala Leu His Met Ala Lys Glu Tyr Val Lys Asn His
115 120 125

Pro Glu Arg Lys Val Leu Val Ile Ala Ser Asp Ile Ala Arg Tyr Gly
130 135 140

Leu Ala Ser Gly Gly Glu Val Thr Gln Gly Val Gly Ala Val Ala Met
145 150 155 160

Met Ile Thr Gln Asn Pro Arg Ile Leu Ser Ile Glu Asp Asp Ser Val
165 170 175

Phe Leu Thr Glu Asp Ile Tyr Asp Phe Trp Arg Pro Asp Tyr Ser Glu
180 185 190

Phe Pro Val Val Asp Gly Pro Leu Ser Asn Ser Thr Tyr Ile Glu Ser
195 200 205

Phe Gln Lys Val Trp Asn Arg His Lys Glu Leu Ser Gly Arg Gly Leu
210 215 220

Glu Asp Tyr Gln Ala Ile Ala Phe His Ile Pro Tyr Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Gln Ser Val Leu Asp Gln Thr Asp Glu Asp Asn Gln
245 250 255

Glu Arg Leu Met Ala Arg Tyr Glu Glu Ser Ile Arg Tyr Ser Arg Arg
260 265 270

Ile Gly Asn Leu Tyr Thr Gly Ser Leu Tyr Leu Gly Leu Thr Ser Leu
275 280 285

Leu Glu Asn Ser Lys Ser Leu Gln Pro Gly Asp Arg Ile Gly Leu Phe
290 295 300

Ser Tyr Gly Ser Gly Ala Val Ser Glu Phe Phe Thr Gly Tyr Leu Glu
305 310 315 320

Glu Asn Tyr Gln Glu Tyr Leu Phe Ala Gln Ser His Gln Glu Met Leu

325 330 335

Asp Ser Arg Thr Arg Ile Thr Val Asp Glu Tyr Glu Thr Ile Phe Ser
340 345 350

Glu Thr Leu Pro Glu His Gly Glu Cys Ala Glu Tyr Thr Ser Asp Val
355 360 365

Pro Phe Ser Ile Thr Lys Ile Glu Asn Asp Ile Arg Tyr Tyr Lys Ile
370 375 380

<210> 78

<211> 388

<212> PRT

<213> Staphylococcus haemolyticus

<400> 78

Met Ser Ile Gly Ile Asp Lys Ile Asn Phe Tyr Val Pro Lys Tyr Tyr
1 5 10 15

Val Asp Met Ala Lys Leu Ala Glu Ala Arg Gln Val Asp Pro Asn Lys
20 25 30

Phe Leu Ile Gly Ile Gly Gln Thr Gln Met Ala Val Ser Pro Val Ser
35 40 45

Gln Asp Ile Val Ser Met Gly Ala Asn Ala Ala Lys Asp Ile Ile Thr
50 55 60

Asp Asp Asp Lys Lys His Ile Gly Met Val Ile Val Ala Thr Glu Ser
65 70 75 80

Ala Ile Asp Asn Ala Lys Ala Ala Ala Val Gln Ile His Asn Leu Leu
85 90 95

Gly Val Gln Pro Phe Ala Arg Cys Phe Glu Met Lys Glu Ala Cys Tyr
100 105 110

Ala Ala Thr Pro Ala Ile Gln Leu Ala Lys Asp Tyr Ile Glu Lys Arg
115 120 125

Pro Asn Glu Lys Val Leu Val Ile Ala Ser Asp Thr Ala Arg Tyr Gly
130 135 140

Ile Gln Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Leu Ile Ser Asn Asn Pro Ser Ile Leu Glu Leu Asn Asp Asp Ala Val
165 170 175

Ala Tyr Thr Glu Asp Val Tyr Asp Phe Trp Arg Pro Thr Gly His Lys

180 185 190

Tyr Pro Leu Val Ala Gly Ala Leu Ser Lys Asp Ala Tyr Ile Lys Ser
195 200 205

Phe Gln Glu Ser Trp Asn Glu Tyr Ala Arg Arg Glu Asp Lys Thr Leu
210 215 220

Ser Asp Phe Glu Ser Leu Cys Phe His Val Pro Phe Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Asp Ser Ile Ile Asn Asp Ala Asp Glu Thr Thr Gln
245 250 255

Glu Arg Leu Thr Ser Gly Tyr Glu Asp Ala Val Tyr Tyr Asn Arg Tyr
260 265 270

Val Gly Asn Ile Tyr Thr Gly Ser Leu Tyr Leu Ser Leu Ile Ser Leu
275 280 285

Leu Glu Asn Arg Ser Leu Lys Gly Gly Gln Thr Ile Gly Leu Phe Ser
290 295 300

Tyr Gly Ser Gly Ser Val Gly Glu Phe Phe Ser Ala Thr Leu Val Glu
305 310 315 320

Gly Tyr Glu Lys Gln Leu Asp Ile Glu Gly His Lys Ala Leu Leu Asn
325 330 335

Glu Arg Gln Glu Val Ser Val Glu Asp Tyr Glu Ser Phe Phe Lys Arg
340 345 350

Phe Asp Asp Leu Glu Phe Asp His Ala Thr Glu Gln Thr Asp Asp Asp
355 360 365

Lys Ser Ile Tyr Tyr Leu Glu Asn Ile Gln Asp Asp Ile Arg Gln Tyr
370 375 380

His Ile Pro Lys
385

<210> 79

<211> 388

<212> PRT

<213> Staphylococcus epidermis

<400> 79

Met Asn Ile Gly Ile Asp Lys Ile Ser Phe Tyr Val Pro Lys Tyr Tyr
1 5 10 15

Val Asp Met Ala Lys Leu Ala Glu Ala Arg Gln Val Asp Pro Asn Lys

20 25 30

Phe Leu Ile Gly Ile Gly Gln Thr Glu Met Thr Val Ser Pro Val Asn
35 40 45

Gln Asp Ile Val Ser Met Gly Ala Asn Ala Ala Lys Asp Ile Ile Thr
50 55 60

Glu Glu Asp Lys Lys Asn Ile Gly Met Val Ile Val Ala Thr Glu Ser
65 70 75 80

Ala Ile Asp Asn Ala Lys Ala Ala Ala Val Gln Ile His His Leu Leu
85 90 95

Gly Ile Gln Pro Phe Ala Arg Cys Phe Glu Met Lys Glu Ala Cys Tyr
100 105 110

Ala Ala Thr Pro Ala Ile Gln Leu Ala Lys Asp Tyr Leu Ala Gln Arg
115 120 125

Pro Asn Glu Lys Val Leu Val Ile Ala Ser Asp Thr Ala Arg Tyr Gly
130 135 140

Ile His Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Met Ile Ser His Asp Pro Ser Ile Leu Lys Leu Asn Asp Asp Ala Val
165 170 175

Ala Tyr Thr Glu Asp Val Tyr Asp Phe Trp Arg Pro Thr Gly His Gln
180 185 190

Tyr Pro Leu Val Ala Gly Ala Leu Ser Lys Asp Ala Tyr Ile Lys Ser
195 200 205

Phe Gln Glu Ser Trp Asn Glu Tyr Ala Arg Arg His Asn Lys Thr Leu
210 215 220

Ala Asp Phe Ala Ser Leu Cys Phe His Val Pro Phe Thr Lys Met Gly
225 230 235 240

Gln Lys Ala Leu Asp Ser Ile Ile Asn His Ala Asp Glu Thr Thr Gln
245 250 255

Asp Arg Leu Asn Ser Ser Tyr Gln Asp Ala Val Asp Tyr Asn Arg Tyr
260 265 270

Val Gly Asn Ile Tyr Thr Gly Ser Leu Tyr Leu Ser Leu Ile Ser Leu
275 280 285

Leu Glu Thr Arg Asp Leu Lys Gly Gly Gln Thr Ile Gly Leu Phe Ser
290 295 300

Tyr Gly Ser Gly Ser Val Gly Glu Phe Phe Ser Gly Thr Leu Val Asp
305 310 315 320

Gly Phe Lys Glu Gln Leu Asp Val Glu Arg His Lys Ser Leu Leu Asn

325 330 335

Asn Arg Ile Glu Val Ser Val Asp Glu Tyr Glu His Phe Phe Lys Arg
340 345 350

Phe Asp Gln Leu Glu Leu Asn His Glu Leu Glu Lys Ser Asn Ala Asp
355 360 365

Arg Asp Ile Phe Tyr Leu Lys Ser Ile Asp Asn Asn Ile Arg Glu Tyr
370 375 380

His Ile Ala Glu
385

<210> 80

<211> 388

<212> PRT

<213> Staphylococcus aureus

<400> 80

Met Thr Ile Gly Ile Asp Lys Ile Asn Phe Tyr Val Pro Lys Tyr Tyr
1 5 10 15

Val Asp Met Ala Lys Leu Ala Glu Ala Arg Gln Val Asp Pro Asn Lys
20 25 30

Phe Leu Ile Gly Ile Gly Gln Thr Glu Met Ala Val Ser Pro Val Asn
35 40 45

Gln Asp Ile Val Ser Met Gly Ala Asn Ala Ala Lys Asp Ile Ile Thr
50 55 60

Asp Glu Asp Lys Lys Lys Ile Gly Met Val Ile Val Ala Thr Glu Ser
65 70 75 80

Ala Val Asp Ala Ala Lys Ala Ala Ala Val Gln Ile His Asn Leu Leu
85 90 95

Gly Ile Gln Pro Phe Ala Arg Cys Phe Glu Met Lys Glu Ala Cys Tyr
100 105 110

Ala Ala Thr Pro Ala Ile Gln Leu Ala Lys Asp Tyr Leu Ala Thr Arg
115 120 125

Pro Asn Glu Lys Val Leu Val Ile Ala Thr Asp Thr Ala Arg Tyr Gly
130 135 140

Leu Asn Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Val Ile Ala His Asn Pro Ser Ile Leu Ala Leu Asn Glu Asp Ala Val

165 170 175

Ala Tyr Thr Glu Asp Val Tyr Asp Phe Trp Arg Pro Thr Gly His Lys
180 185 190

Tyr Pro Leu Val Asp Gly Ala Leu Ser Lys Asp Ala Tyr Ile Arg Ser
195 200 205

Phe Gln Gln Ser Trp Asn Glu Tyr Ala Lys Arg Gln Gly Lys Ser Leu
210 215 220

Ala Asp Phe Ala Ser Leu Cys Phe His Val Pro Phe Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Glu Ser Ile Ile Asp Asn Ala Asp Glu Thr Thr Gln
245 250 255

Glu Arg Leu Arg Ser Gly Tyr Glu Asp Ala Val Asp Tyr Asn Arg Tyr
260 265 270

Val Gly Asn Ile Tyr Thr Gly Ser Leu Tyr Leu Ser Leu Ile Ser Leu
275 280 285

Leu Glu Asn Arg Asp Leu Gln Ala Gly Glu Thr Ile Gly Leu Phe Ser
290 295 300

Tyr Gly Ser Gly Ser Val Val Glu Phe Tyr Ser Ala Thr Leu Val Val
305 310 315 320

Gly Tyr Lys Asp His Leu Asp Gln Ala Ala His Lys Ala Leu Leu Asn
325 330 335

Asn Arg Thr Glu Val Ser Val Asp Ala Tyr Glu Thr Phe Phe Lys Arg
340 345 350

Phe Asp Asp Val Glu Phe Asp Glu Glu Gln Asp Ala Val His Glu Asp
355 360 365

Arg His Ile Phe Tyr Leu Ser Asn Ile Glu Asn Asn Val Arg Glu Tyr
370 375 380

His Arg Pro Glu
385

<210> 81

<211> 389

<212> PRT

<213> Staphylococcus carnosus

<400> 81

Met Thr Ile Gly Ile Asp Gln Leu Asn Phe Tyr Ile Pro Asn Phe Tyr

1 5 10 15

Val Asp Met Ala Glu Leu Ala Glu Ala Arg Gly Val Asp Pro Asn Lys
20 25 30

Phe Leu Ile Gly Ile Gly Gln Ser Gln Met Ala Val Ser Pro Val Ser
35 40 45

Gln Asp Ile Val Ser Met Gly Ala Asn Ala Ala Gln Pro Ile Leu Ser
50 55 60

Glu Gln Asp Lys Lys Asp Ile Thr Met Val Ile Val Ala Thr Glu Ser
65 70 75 80

Ala Ile Asp Ser Ala Lys Ala Ser Ala Val Gln Ile His His Leu Leu
85 90 95

Gly Ile Gln Pro Phe Ala Arg Cys Phe Glu Met Lys Glu Ala Cys Tyr
100 105 110

Ala Ala Thr Pro Ala Ile Gln Leu Ala Lys Asp Tyr Leu Val Pro Arg
115 120 125

Pro Lys Glu Lys Val Leu Val Ile Ala Ser Asp Thr Ala Arg Tyr Gly
130 135 140

Leu Asn Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Val Ile Ser His Asn Pro Ser Ile Leu Glu Leu His Asp Asp Ser Val
165 170 175

Ala Tyr Thr Glu Asp Val Tyr Asp Phe Trp Arg Pro Ser Gly Glu Ile
180 185 190

Tyr Pro Leu Val Ala Gly Lys Leu Ser Lys Asp Ala Tyr Ile Lys Ser
195 200 205

Phe Gln Glu Ser Trp Asn Glu Tyr Ala Lys Arg His His Lys Ser Leu
210 215 220

Ser Asp Phe Ala Ala Leu Cys Phe His Val Pro Phe Thr Lys Met Gly
225 230 235 240

Gln Lys Ala Leu Asp Ser Ile Leu Thr Asp Ser Ala Ser Glu Asp Thr
245 250 255

Gln Ala Arg Leu Asn Glu Gly Tyr Lys Ser Ala Thr Asp Tyr Asn Arg
260 265 270

Tyr Val Gly Asn Val Tyr Thr Gly Ser Leu Tyr Leu Ser Leu Ile Ser
275 280 285

Leu Leu Glu Asn His Lys Leu Asn Gly Gly Asp Asn Ile Gly Leu Phe
290 295 300

Ser Tyr Gly Ser Gly Ser Val Gly Glu Phe Phe Ser Ala Thr Leu Val

305 310 315 320

Asp Asn Tyr Gln Asp His Leu Asp Val Lys Ala His Lys Ala Met Leu
325 330 335

Asp Asn Arg Lys Ala Leu Ser Val Glu Glu Tyr Glu Lys Phe Phe Asn
340 345 350

Arg Phe Asp Asn Leu Glu Phe Asp Thr Glu Thr Glu Leu Glu Val Glu
355 360 365

Pro Lys Gly Asn Phe Tyr Leu Lys Glu Ile Ser Asp Asn Ile Arg Tyr
370 375 380

Tyr Asp Thr Val Lys
385

<210> 82

<211> 389

<212> PRT

<213> Streptomyces sp. CL190

<400> 82

Met Ser Ile Ser Ile Gly Ile His Asp Leu Ser Phe Ala Thr Thr Glu
1 5 10 15

Phe Val Leu Pro His Thr Ala Leu Ala Glu Tyr Asn Gly Thr Glu Ile
20 25 30

Gly Lys Tyr His Val Gly Ile Gly Gln Gln Ser Met Ser Val Pro Ala
35 40 45

Ala Asp Glu Asp Ile Val Thr Met Ala Ala Thr Ala Ala Arg Pro Ile
50 55 60

Ile Glu Arg Asn Gly Lys Ser Arg Ile Arg Thr Val Val Phe Ala Thr
65 70 75 80

Glu Ser Ser Ile Asp Gln Ala Lys Ala Gly Gly Val Tyr Val His Ser
85 90 95

Leu Leu Gly Leu Glu Ser Ala Cys Arg Val Val Glu Leu Lys Gln Ala
100 105 110

Cys Tyr Gly Ala Thr Ala Ala Leu Gln Phe Ala Ile Gly Leu Val Arg
115 120 125

Arg Asp Pro Ala Gln Gln Val Leu Val Ile Ala Ser Asp Val Ser Lys
130 135 140

Tyr Glu Leu Asp Ser Pro Gly Glu Ala Thr Gln Gly Ala Ala Ala Val

145 150 155 160

Ala Met Leu Val Gly Ala Asp Pro Ala Leu Leu Arg Ile Glu Glu Pro
165 170 175

Ser Gly Leu Phe Thr Ala Asp Val Met Asp Phe Trp Arg Pro Asn Tyr
180 185 190

Leu Thr Thr Ala Leu Val Asp Gly Gln Glu Ser Ile Asn Ala Tyr Leu
195 200 205

Gln Ala Val Glu Gly Ala Trp Lys Asp Tyr Ala Glu Gln Asp Gly Arg
210 215 220

Ser Leu Glu Glu Phe Ala Ala Phe Val Tyr His Gln Pro Phe Thr Lys
225 230 235 240

Met Ala Tyr Lys Ala His Arg His Leu Leu Asn Phe Asn Gly Tyr Asp
245 250 255

Thr Asp Lys Asp Ala Ile Glu Gly Ala Leu Gly Gln Thr Thr Ala Tyr
260 265 270

Asn Asn Val Ile Gly Asn Ser Tyr Thr Ala Ser Val Tyr Leu Gly Leu
275 280 285

Ala Ala Leu Leu Asp Gln Ala Asp Asp Leu Thr Gly Arg Ser Ile Gly
290 295 300

Phe Leu Ser Tyr Gly Ser Gly Ser Val Ala Glu Phe Phe Ser Gly Thr
305 310 315 320

Val Val Ala Gly Tyr Arg Glu Arg Leu Arg Thr Glu Ala Asn Gln Glu
325 330 335

Ala Ile Ala Arg Arg Lys Ser Val Asp Tyr Ala Thr Tyr Arg Glu Leu
340 345 350

His Glu Tyr Thr Leu Pro Ser Asp Gly Gly Asp His Ala Thr Pro Val
355 360 365

Gln Thr Thr Gly Pro Phe Arg Leu Ala Gly Ile Asn Asp His Lys Arg
370 375 380

Ile Tyr Glu Ala Arg
385

<210> 83

<211> 389

<212> PRT

<213> Streptomyces griseolosporeus

<400> 83

Met Pro Leu Ala Ile Gly Ile His Asp Leu Ser Phe Ala Thr Gly Glu
1   5   10   15

Phe Val Leu Pro His Thr Ala Leu Ala Ala His Asn Gly Thr Glu Ile
            20   25   30

Gly Lys Tyr His Ala Gly Ile Gly Gln Glu Ser Met Ser Val Pro Ala
        35   40   45

Ala Asp Glu Asp Ile Val Thr Leu Ala Ala Thr Ala Ala Ala Pro Ile
    50   55   60

Val Ala Arg His Gly Ser Asp Arg Ile Arg Thr Val Val Leu Ala Thr
65   70   75   80

Glu Ser Ser Ile Asp Gln Ala Lys Ser Ala Gly Val Tyr Val His Ser
                85   90   95

Leu Leu Gly Leu Pro Ser Ala Thr Arg Val Val Glu Leu Lys Gln Ala
            100   105   110

Cys Tyr Gly Ala Thr Ala Gly Leu Gln Phe Ala Ile Gly Leu Val Gln
        115   120   125

Arg Asp Pro Ala Gln Gln Val Leu Val Ile Ala Ser Asp Val Ser Lys
    130   135   140

Tyr Asp Leu Asp Ser Pro Gly Glu Ala Thr Gln Gly Ala Ala Ala Val
145   150   155   160

Ala Met Leu Val Gly Ala Asp Pro Gly Leu Val Arg Ile Glu Asp Pro
                165   170   175

Ser Gly Leu Phe Thr Val Asp Val Met Asp Phe Trp Arg Pro Asn Tyr
            180   185   190

Arg Thr Thr Ala Leu Val Asp Gly Gln Glu Ser Ile Gly Ala Tyr Leu
        195   200   205

Gln Ala Val Glu Gly Ala Trp Lys Asp Tyr Ser Glu Arg Gly Gly His
    210   215   220

Ser Leu Glu Gln Phe Ala Ala Phe Cys Tyr His Gln Pro Phe Thr Lys
225   230   235   240

Met Ala His Lys Ala His Arg His Leu Leu Asn Tyr Cys Ser His Asp
                245   250   255

Ile His His Asp Asp Val Thr Arg Ala Val Gly Arg Thr Thr Ala Tyr
            260   265   270

Asn Arg Val Ile Gly Asn Ser Tyr Thr Ala Ser Val Tyr Leu Gly Leu
        275   280   285

Ala Ala Leu Leu Asp Gln Ala Asp Asp Leu Thr Gly Glu Arg Ile Gly

290 295 300

Phe Leu Ser Tyr Gly Ser Gly Ser Val Ala Glu Phe Phe Gly Gly Ile
305 310 315 320

Val Val Ala Gly Tyr Arg Asp Arg Leu Arg Thr Ala Ala Asn Ile Glu
325 330 335

Ala Val Ser Arg Arg Arg Pro Ile Asp Tyr Ala Gly Tyr Arg Glu Leu
340 345 350

His Glu Trp Ala Phe Pro Ala Arg Arg Gly Ala His Ser Thr Pro Gln
355 360 365

Gln Thr Thr Gly Pro Phe Arg Leu Ser Gly Ile Ser Gly His Lys Arg
370 375 380

Leu Tyr Arg Ala Cys
385

<210> 84

<211> 407

<212> PRT

<213> Borrelia burgdorferi

<400> 84

Met Arg Ile Gly Ile Ser Asp Ile Arg Ile Phe Leu Pro Leu Asn Tyr
1 5 10 15

Leu Asp Phe Ser Val Leu Leu Glu Asn Pro Leu Tyr Phe Ser Asn Glu
20 25 30

Val Phe Phe Lys Lys Ile Asn Arg Ala Ile Asp Ala Thr Leu Gln Lys
35 40 45

Gly Phe Arg Phe Thr Ser Pro Asn Glu Asp Ser Val Thr Met Ala Ser
50 55 60

Ser Ala Val Lys Leu Ile Phe Asp Asn Asn Asn Leu Asp Leu Ser Lys
65 70 75 80

Ile Arg Ile Leu Leu Gly Gly Thr Glu Thr Gly Val Asp His Ser Lys
85 90 95

Ala Ile Ser Ser Tyr Val Phe Gly Ala Leu Lys Gln Ser Gly Ile Cys
100 105 110

Leu Gly Asn Asn Phe Leu Thr Phe Gln Val Gln His Ala Cys Ala Gly
115 120 125

Ala Ala Met Ser Leu His Thr Val Ala Ser Val Leu Ser His Ser Asn

130 135 140

Asn Ser Glu Tyr Gly Ile Val Phe Ser Ser Asp Ile Ala His Tyr Ser
145 150 155 160

Asn Leu Thr Thr Ala Glu Ile Thr Gln Gly Ala Gly Ala Thr Ala Ile
165 170 175

Leu Ile Glu Lys Asn Pro Lys Ile Leu Ser Ile Asn Leu Ser Glu Phe
180 185 190

Gly Val Tyr Thr Asp Asp Val Asp Asp Phe Phe Arg Pro Phe Gly Ser
195 200 205

Val Glu Ala Lys Val Arg Gly Gln Tyr Ser Val Glu Cys Tyr Asn Asn
210 215 220

Ala Asn Glu Asn Ala Leu Arg Asp Phe Ala Phe Lys Lys Gln Leu Ser
225 230 235 240

Met Lys Asp Leu Phe Ser Asn Tyr Arg Phe Val Leu His Val Pro Phe
245 250 255

Ala Lys Met Pro Ile Asp Ser Met His Tyr Ile Leu Lys Lys Tyr Tyr
260 265 270

Ser Asp Asp Glu Ser Val Arg Asn Ala Tyr Leu Glu Ser Ile Asp Phe
275 280 285

Tyr Asp Gly Val Glu Ala Ala Met Glu Val Gly Asn Leu Tyr Thr Gly
290 295 300

Ser Ile Phe Leu Ser Leu Ala Phe Tyr Leu Lys Arg Val Phe Ser Lys
305 310 315 320

Lys Asp Ile Thr Gly Glu Lys Ile Leu Phe Cys Ser Tyr Gly Ser Gly
325 330 335

Asn Ile Met Ile Ile Tyr Glu Leu Thr Ile Glu Lys Ser Ala Phe Asp
340 345 350

Val Ile Lys Leu Trp Asp Leu Glu Gly Leu Ile Lys Asn Arg Asn Asn
355 360 365

Ala Asn Phe Glu Glu Tyr Lys Asp Phe Phe Gln Asn Lys Ile Ile Pro
370 375 380

Gly Glu Ser Arg Gly Phe Tyr Leu Lys Glu Leu Arg Asn Asp Gly Tyr
385 390 395 400

Arg Val Tyr Gly Tyr Arg Ala
405

<210> 85

<211> 317

<212> PRT

<213> Streptococcus pneumoniae

<400> 85

Met Asp Arg Glu Pro Val Thr Val Arg Ser Tyr Ala Asn Ile Ala Ile
1 5 10 15

Ile Lys Tyr Trp Gly Lys Lys Lys Glu Lys Glu Met Val Pro Ala Thr
20 25 30

Ser Ser Ile Ser Leu Thr Leu Glu Asn Met Tyr Thr Glu Thr Thr Leu
35 40 45

Ser Pro Leu Pro Ala Asn Val Thr Ala Asp Glu Phe Tyr Ile Asn Gly
50 55 60

Gln Leu Gln Asn Glu Val Glu His Ala Lys Met Ser Lys Ile Ile Asp
65 70 75 80

Arg Tyr Arg Pro Ala Gly Glu Gly Phe Val Arg Ile Asp Thr Gln Asn
85 90 95

Asn Met Pro Thr Ala Ala Gly Leu Ser Ser Ser Ser Ser Gly Leu Ser
100 105 110

Ala Leu Val Lys Ala Cys Asn Ala Tyr Phe Lys Leu Gly Leu Asp Arg
115 120 125

Ser Gln Leu Ala Gln Glu Ala Lys Phe Ala Ser Gly Ser Ser Ser Arg
130 135 140

Ser Phe Tyr Gly Pro Leu Gly Ala Trp Asp Lys Asp Ser Gly Glu Ile
145 150 155 160

Tyr Pro Val Glu Thr Asp Leu Lys Leu Ala Met Ile Met Leu Val Leu
165 170 175

Glu Asp Lys Lys Lys Pro Ile Ser Ser Arg Asp Gly Met Lys Leu Cys
180 185 190

Val Glu Thr Ser Thr Thr Phe Asp Asp Trp Val Arg Gln Ser Glu Lys
195 200 205

Asp Tyr Gln Asp Met Leu Ile Tyr Leu Lys Glu Asn Asp Phe Ala Lys
210 215 220

Ile Gly Glu Leu Thr Glu Lys Asn Ala Leu Ala Met His Ala Thr Thr
225 230 235 240

Lys Thr Ala Ser Pro Ala Phe Ser Tyr Leu Thr Asp Ala Ser Tyr Glu
245 250 255

Ala Met Ala Phe Val Arg Gln Leu Arg Glu Lys Gly Glu Ala Cys Tyr

260 265 270

Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Phe Cys Gln Glu Lys
275 280 285

Asp Leu Glu His Leu Ser Glu Ile Phe Gly Gln Arg Tyr Arg Leu Ile
290 295 300

Val Ser Lys Thr Lys Asp Leu Ser Gln Asp Asp Cys Cys
305 310 315

<210> 86

<211> 314

<212> PRT

<213> Streptococcus pyrogenes

<400> 86

Met Asp Pro Asn Val Ile Thr Val Thr Ser Tyr Ala Asn Ile Ala Ile
1 5 10 15

Ile Lys Tyr Trp Gly Lys Glu Asn Gln Ala Lys Met Ile Pro Ser Thr
20 25 30

Ser Ser Ile Ser Leu Thr Leu Glu Asn Met Phe Thr Thr Thr Ser Val
35 40 45

Ser Phe Leu Pro Asp Thr Ala Thr Ser Asp Gln Phe Tyr Ile Asn Gly
50 55 60

Val Leu Gln Asn Asp Glu Glu His Thr Lys Ile Ser Thr Ile Ile Asp
65 70 75 80

Gln Phe Arg Gln Pro Gly Gln Ala Phe Val Lys Met Glu Thr Gln Asn
85 90 95

Asn Met Pro Thr Ala Ala Gly Leu Ser Ser Ser Ser Ser Gly Leu Ser
100 105 110

Ala Leu Val Lys Ala Cys Asp Gln Leu Phe Asp Thr Gln Leu Asp Gln
115 120 125

Lys Ala Leu Ala Gln Lys Ala Lys Phe Ala Ser Gly Ser Ser Ser Arg
130 135 140

Ser Phe Phe Gly Pro Val Ala Ala Trp Asp Lys Asp Ser Gly Ala Ile
145 150 155 160

Tyr Lys Val Glu Thr Asp Leu Lys Met Ala Met Ile Met Leu Val Leu
165 170 175

Asn Ala Ala Lys Lys Pro Ile Ser Ser Arg Glu Gly Met Lys Leu Cys

180 185 190

Arg Asp Thr Ser Thr Thr Phe Asp Glu Trp Val Glu Gln Ser Ala Ile
195 200 205

Asp Tyr Gln His Met Leu Thr Tyr Leu Lys Thr Asn Asn Phe Glu Lys
210 215 220

Val Gly Gln Leu Thr Glu Ala Asn Ala Leu Ala Met His Ala Thr Thr
225 230 235 240

Lys Thr Ala Asn Pro Pro Phe Ser Tyr Leu Thr Lys Glu Ser Tyr Gln
245 250 255

Ala Met Glu Ala Val Lys Glu Leu Arg Gln Glu Gly Phe Ala Cys Tyr
260 265 270

Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Leu Cys Leu Glu Lys
275 280 285

Asp Leu Ala Gln Leu Ala Glu Arg Leu Gly Lys Asn Tyr Arg Ile Ile
290 295 300

Val Ser Lys Thr Lys Asp Leu Pro Asp Val
305 310

<210> 87

<211> 331

<212> PRT

<213> Enterococcus faecalis

<400> 87

Met Leu Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile Lys
1 5 10 15

Tyr Trp Gly Lys Ala Asn Glu Glu Tyr Ile Leu Pro Met Asn Ser Ser
20 25 30

Leu Ser Leu Thr Leu Asp Ala Phe Tyr Thr Glu Thr Thr Val Thr Phe
35 40 45

Asp Ala His Tyr Ser Glu Asp Val Phe Ile Leu Asn Gly Ile Leu Gln
50 55 60

Asn Glu Lys Gln Thr Lys Lys Val Lys Glu Phe Leu Asn Leu Val Arg
65 70 75 80

Gln Gln Ala Asp Cys Thr Trp Phe Ala Lys Val Glu Ser Gln Asn Phe
85 90 95

Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Leu Ala Ala

100 105 110

Leu Ala Gly Ala Cys Asn Val Ala Leu Gly Leu Asn Leu Ser Ala Lys
115 120 125

Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Cys Arg Ser
130 135 140

Ile Phe Gly Gly Phe Ala Gln Trp Asn Lys Gly His Ser Asp Glu Thr
145 150 155 160

Ser Phe Ala Glu Asn Ile Pro Ala Asn Asn Trp Glu Asn Glu Leu Ala
165 170 175

Met Leu Phe Ile Leu Ile Asn Asp Gly Glu Lys Asp Val Ser Ser Arg
180 185 190

Asp Gly Met Lys Arg Thr Val Glu Thr Ser Ser Phe Tyr Gln Gly Trp
195 200 205

Leu Asp Asn Val Glu Lys Asp Leu Ser Gln Val His Glu Ala Ile Lys
210 215 220

Thr Lys Asp Phe Pro Arg Leu Gly Glu Ile Ile Glu Ala Asn Gly Leu
225 230 235 240

Arg Met His Gly Thr Thr Leu Gly Ala Val Pro Pro Phe Thr Tyr Trp
245 250 255

Ser Pro Gly Ser Leu Gln Ala Met Ala Leu Val Arg Gln Ala Arg Ala
260 265 270

Lys Gly Ile Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val Lys
275 280 285

Val Leu Val Glu Lys Lys Asn Leu Glu Ala Leu Lys Thr Phe Leu Ser
290 295 300

Glu His Phe Ser Lys Glu Gln Leu Val Pro Ala Phe Ala Gly Pro Gly
305 310 315 320

Ile Glu Leu Phe Glu Thr Lys Gly Met Asp Lys
325 330

<210> 88

<211> 325

<212> PRT

<213> Enterococcus faecium

<400> 88

Met Phe Lys Gly Lys Ala Arg Ala Tyr Thr Asn Ile Ala Leu Ile Lys

1 5 10 15

Tyr Trp Gly Lys Lys Asn Glu Glu Leu Ile Leu Pro Met Asn Asn Ser
20 25 30

Leu Ser Leu Thr Leu Asp Ala Phe Tyr Thr Glu Thr Glu Val Ile Phe
35 40 45

Ser Asp Ser Tyr Met Val Asp Glu Phe Tyr Leu Asp Gly Thr Leu Gln
50 55 60

Asp Glu Lys Ala Thr Lys Lys Val Ser Gln Phe Leu Asp Leu Phe Arg
65 70 75 80

Lys Glu Ala Gly Leu Ser Leu Lys Ala Ser Val Ile Ser Gln Asn Phe
85 90 95

Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Gly Leu Ala Ala
100 105 110

Leu Ala Gly Ala Cys Asn Thr Ala Leu Lys Leu Gly Leu Asp Asp Leu
115 120 125

Ser Leu Ser Arg Phe Ala Arg Arg Gly Ser Gly Ser Ala Cys Arg Ser
130 135 140

Ile Phe Gly Gly Phe Val Glu Trp Glu Lys Gly His Asp Asp Leu Ser
145 150 155 160

Ser Tyr Ala Lys Pro Val Pro Ser Asp Ser Phe Glu Asp Asp Leu Ala
165 170 175

Met Val Phe Val Leu Ile Asn Asp Gln Lys Lys Glu Val Ser Ser Arg
180 185 190

Asn Gly Met Arg Arg Thr Val Glu Thr Ser Asn Phe Tyr Gln Gly Trp
195 200 205

Leu Asp Ser Val Glu Gly Asp Leu Tyr Gln Leu Lys Gln Ala Ile Lys
210 215 220

Thr Lys Asp Phe Gln Leu Leu Gly Glu Thr Met Glu Arg Asn Gly Leu
225 230 235 240

Lys Met His Gly Thr Thr Leu Ala Ala Gln Pro Pro Phe Thr Tyr Trp
245 250 255

Ser Pro Asn Ser Leu Lys Ala Met Asp Ala Val Arg Gln Leu Arg Lys
260 265 270

Gln Gly Ile Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val Lys
275 280 285

Val Leu Val Glu Asn Ser His Leu Ser Glu Val Gln Glu Thr Phe Thr
290 295 300

Lys Leu Phe Ser Lys Glu Gln Val Ile Thr Ala His Ala Gly Pro Gly

305 310 315 320

Ile Ala Ile Ile Glu
325

<210> 89

<211> 327

<212> PRT

<213> Staphylococcus haemolyticus

<400> 89

Met Lys Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile
1 5 10 15

Lys Tyr Trp Gly Lys Ala Asp Glu Ala Leu Ile Ile Pro Met Asn Asn
20 25 30

Ser Leu Ser Val Thr Leu Asp Arg Phe Tyr Thr Glu Thr Arg Val Thr
35 40 45

Phe Asp Glu Thr Leu Thr Glu Asp Gln Leu Ile Leu Asn Gly Glu Ala
50 55 60

Val Asn Ala Lys Glu Ser Ala Lys Ile Gln Arg Tyr Met Glu Met Ile
65 70 75 80

Arg Lys Glu Ala Gly Ile Ser His Glu Ala Leu Ile Glu Ser Glu Asn
85 90 95

Phe Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala
100 105 110

Ala Leu Ala Gly Ala Cys Asn Glu Ala Leu Gln Leu Gly Leu Ser Asp
115 120 125

Lys Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg
130 135 140

Ser Ile Tyr Gly Gly Phe Ala Glu Trp Glu Lys Gly Asn Asp Asp Glu
145 150 155 160

Thr Ser Phe Ala His Arg Val Glu Ala Asp Gly Trp Glu Asn Glu Leu
165 170 175

Ala Met Val Phe Val Val Ile Asn Asn Lys Ser Lys Lys Val Ser Ser
180 185 190

Arg Ser Gly Met Ser Leu Thr Arg Asp Thr Ser Arg Phe Tyr Gln Tyr
195 200 205

Trp Leu Asp Asn Val Glu Pro Asp Leu Lys Glu Thr Lys Glu Ala Ile

210 215 220

Ala Gln Lys Asp Phe Lys Arg Met Gly Glu Val Ile Glu Ala Asn Gly
225 230 235 240

Leu Arg Met His Ala Thr Asn Leu Gly Ala Gln Pro Pro Phe Thr Tyr
245 250 255

Leu Val Pro Glu Ser Tyr Asp Ala Met Arg Ile Val His Glu Cys Arg
260 265 270

Glu Ala Gly Leu Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val
275 280 285

Lys Val Leu Ile Glu Lys Lys Asn Gln Gln Ala Ile Val Asp Lys Phe
290 295 300

Leu Gln Glu Phe Asp Gln Ser Gln Ile Ile Thr Ser Asp Ile Thr Gln
305 310 315 320

Ser Gly Val Glu Ile Ile Lys
325

<210> 90

<211> 327

<212> PRT

<213> Staphylococcus epidermis

<400> 90

Met Val Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile
1 5 10 15

Lys Tyr Trp Gly Lys Ala Asp Glu Thr Tyr Ile Ile Pro Met Asn Asn
20 25 30

Ser Leu Ser Val Thr Leu Asp Arg Phe Tyr Thr Glu Thr Lys Val Thr
35 40 45

Phe Asp Pro Asp Phe Thr Glu Asp Cys Leu Ile Leu Asn Gly Asn Glu
50 55 60

Val Asn Ala Lys Glu Lys Glu Lys Ile Gln Asn Tyr Met Asn Ile Val
65 70 75 80

Arg Asp Leu Ala Gly Asn Arg Leu His Ala Arg Ile Glu Ser Glu Asn
85 90 95

Tyr Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala
100 105 110

Ala Leu Ala Ala Ala Cys Asn Glu Ala Leu Ser Leu Asn Leu Ser Asp

115 120 125

Thr Asp Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg
130 135 140

Ser Ile Phe Gly Gly Phe Ala Glu Trp Glu Lys Gly His Asp Asp Leu
145 150 155 160

Thr Ser Tyr Ala His Gly Ile Asn Ser Asn Gly Trp Glu Lys Asp Leu
165 170 175

Ser Met Ile Phe Val Val Ile Asn Asn Gln Ser Lys Lys Val Ser Ser
180 185 190

Arg Ser Gly Met Ser Leu Thr Arg Asp Thr Ser Arg Phe Tyr Gln Tyr
195 200 205

Trp Leu Asp His Val Asp Glu Asp Leu Asn Glu Ala Lys Glu Ala Val
210 215 220

Lys Asn Gln Asp Phe Gln Arg Leu Gly Glu Val Ile Glu Ala Asn Gly
225 230 235 240

Leu Arg Met His Ala Thr Asn Leu Gly Ala Gln Pro Pro Phe Thr Tyr
245 250 255

Leu Val Gln Glu Ser Tyr Asp Ala Met Ala Ile Val Glu Gln Cys Arg
260 265 270

Lys Ala Asn Leu Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val
275 280 285

Lys Val Leu Val Glu Lys Lys Asn Lys Gln Ala Val Met Glu Gln Phe
290 295 300

Leu Lys Val Phe Asp Glu Ser Lys Ile Ile Ala Ser Asp Ile Ile Ser
305 310 315 320

Ser Gly Val Glu Ile Ile Lys
325

<210> 91

<211> 327

<212> PRT

<213> Staphylococcus aureus

<400> 91

Met Ile Lys Ser Gly Lys Ala Arg Ala His Thr Asn Ile Ala Leu Ile
1 5 10 15

Lys Tyr Trp Gly Lys Lys Asp Glu Ala Leu Ile Ile Pro Met Asn Asn

20 25 30

Ser Ile Ser Val Thr Leu Glu Lys Phe Tyr Thr Glu Thr Lys Val Thr
35 40 45

Phe Asn Asp Gln Leu Thr Gln Asp Gln Phe Trp Leu Asn Gly Glu Lys
50 55 60

Val Ser Gly Lys Glu Leu Glu Lys Ile Ser Lys Tyr Met Asp Ile Val
65 70 75 80

Arg Asn Arg Ala Gly Ile Asp Trp Tyr Ala Glu Ile Glu Ser Asp Asn
85 90 95

Phe Val Pro Thr Ala Ala Gly Leu Ala Ser Ser Ala Ser Ala Tyr Ala
100 105 110

Ala Leu Ala Ala Ala Cys Asn Gln Ala Leu Asp Leu Gln Leu Ser Asp
115 120 125

Lys Asp Leu Ser Arg Leu Ala Arg Ile Gly Ser Gly Ser Ala Ser Arg
130 135 140

Ser Ile Tyr Gly Gly Phe Ala Glu Trp Glu Lys Gly Tyr Asn Asp Glu
145 150 155 160

Thr Ser Tyr Ala Val Pro Leu Glu Ser Asn His Phe Glu Asp Asp Leu
165 170 175

Ala Met Ile Phe Val Val Ile Asn Gln His Ser Lys Lys Val Pro Ser
180 185 190

Arg Tyr Gly Met Ser Leu Thr Arg Asn Thr Ser Arg Phe Tyr Gln Tyr
195 200 205

Trp Leu Asp His Ile Asp Glu Asp Leu Ala Glu Ala Lys Ala Ala Ile
210 215 220

Gln Asp Lys Asp Phe Lys Arg Leu Gly Glu Val Ile Glu Glu Asn Gly
225 230 235 240

Leu Arg Met His Ala Thr Asn Leu Gly Ser Thr Pro Pro Phe Thr Tyr
245 250 255

Leu Val Gln Glu Ser Tyr Asp Val Met Ala Leu Val His Glu Cys Arg
260 265 270

Glu Ala Gly Tyr Pro Cys Tyr Phe Thr Met Asp Ala Gly Pro Asn Val
275 280 285

Lys Ile Leu Val Glu Lys Lys Asn Lys Gln Gln Ile Ile Asp Lys Leu
290 295 300

Leu Thr Gln Phe Asp Asn Asn Gln Ile Ile Asp Ser Asp Ile Ile Ala
305 310 315 320

Thr Gly Ile Glu Ile Ile Glu

325

<210> 92

<211> 350

<212> PRT

<213> Streptomyces sp. CL190

<400> 92

Met Arg Ser Glu His Pro Thr Thr Thr Val Leu Gln Ser Arg Glu Gln
1 5 10 15

Gly Ser Ala Ala Gly Ala Thr Ala Val Ala His Pro Asn Ile Ala Leu
20 25 30

Ile Lys Tyr Trp Gly Lys Arg Asp Glu Arg Leu Ile Leu Pro Cys Thr
35 40 45

Thr Ser Leu Ser Met Thr Leu Asp Val Phe Pro Thr Thr Thr Glu Val
50 55 60

Arg Leu Asp Pro Ala Ala Glu His Asp Thr Ala Ala Leu Asn Gly Glu
65 70 75 80

Val Ala Thr Gly Glu Thr Leu Arg Arg Ile Ser Ala Phe Leu Ser Leu
85 90 95

Val Arg Glu Val Ala Gly Ser Asp Gln Arg Ala Val Val Asp Thr Arg
100 105 110

Asn Thr Val Pro Thr Gly Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe
115 120 125

Ala Ala Leu Ala Val Ala Ala Ala Ala Ala Tyr Gly Leu Glu Leu Asp
130 135 140

Asp Arg Gly Leu Ser Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser
145 150 155 160

Arg Ser Ile Phe Gly Gly Phe Ala Val Trp His Ala Gly Pro Asp Gly
165 170 175

Thr Ala Thr Glu Ala Asp Leu Gly Ser Tyr Ala Glu Pro Val Pro Ala
180 185 190

Ala Asp Leu Asp Pro Ala Leu Val Ile Ala Val Val Asn Ala Gly Pro
195 200 205

Lys Pro Val Ser Ser Arg Glu Ala Met Arg Arg Thr Val Asp Thr Ser
210 215 220

Pro Leu Tyr Arg Pro Trp Ala Asp Ser Ser Lys Asp Asp Leu Asp Glu

225 230 235 240

Met Arg Ser Ala Leu Leu Arg Gly Asp Leu Glu Ala Val Gly Glu Ile
245 250 255

Ala Glu Arg Asn Ala Leu Gly Met His Ala Thr Met Leu Ala Ala Arg
260 265 270

Pro Ala Val Arg Tyr Leu Ser Pro Ala Thr Val Thr Val Leu Asp Ser
275 280 285

Val Leu Gln Leu Arg Lys Asp Gly Val Leu Ala Tyr Ala Thr Met Asp
290 295 300

Ala Gly Pro Asn Val Lys Val Leu Cys Arg Arg Ala Asp Ala Glu Arg
305 310 315 320

Val Ala Asp Val Val Arg Ala Ala Ala Ser Gly Gly Gln Val Leu Val
325 330 335

Ala Gly Pro Gly Asp Gly Ala Arg Leu Leu Ser Glu Gly Ala
340 345 350

<210> 93

<211> 331

<212> PRT

<213> Streptomyces griseolosporeus

<400> 93

Ala Thr Ala Val Ala Gln Pro Asn Ile Ala Leu Ile Lys Tyr Trp Gly
1 5 10 15

Lys Lys Asp Glu His Leu Val Leu Pro Arg Thr Asp Ser Leu Ser Met
20 25 30

Thr Leu Asp Ile Phe Pro Thr Thr Thr Arg Val Gln Leu Ala Pro Gly
35 40 45

Ala Gly Gln Asp Thr Val Ala Phe Asn Gly Glu Pro Ala Thr Gly Glu
50 55 60

Ala Glu Arg Arg Ile Thr Ala Phe Leu Arg Leu Val Arg Glu Arg Ser
65 70 75 80

Gly Arg Thr Glu Arg Ala Arg Val Glu Thr Glu Asn Thr Val Pro Thr
85 90 95

Gly Ala Gly Leu Ala Ser Ser Ala Ser Gly Phe Ala Ala Leu Ala Val
100 105 110

Ala Ala Ala Ala Ala Tyr Gly Leu Gly Leu Asp Ala Arg Gly Leu Ser

115 120 125

Arg Leu Ala Arg Arg Gly Ser Gly Ser Ala Ser Arg Ser Ile Phe Asp
130 135 140

Gly Phe Ala Val Trp His Ala Gly His Ala Gly Gly Thr Pro Glu Glu
145 150 155 160

Ala Asp Leu Gly Ser Tyr Ala Glu Pro Val Pro Ala Val Asp Leu Glu
165 170 175

Pro Ala Leu Val Val Ala Val Val Ser Ala Ala Pro Lys Ala Val Ser
180 185 190

Ser Arg Glu Ala Met Arg Arg Thr Val Asp Thr Ser Pro Leu Tyr Glu
195 200 205

Pro Trp Ala Val Ser Ser Arg Ala Asp Leu Ala Asp Ile Gly Ala Ala
210 215 220

Leu Ala Arg Gly Asn Leu Pro Ala Val Gly Glu Ile Ala Glu Arg Asn
225 230 235 240

Ala Leu Gly Met His Ala Thr Met Leu Ala Ala Arg Pro Ala Val Arg
245 250 255

Tyr Leu Ser Pro Ala Ser Leu Ala Val Leu Asp Gly Val Leu Gln Leu
260 265 270

Arg Arg Asp Gly Val Pro Ala Tyr Ala Thr Met Asp Ala Gly Pro Asn
275 280 285

Val Lys Val Leu Cys Pro Arg Ser Asp Ala Glu Arg Val Ala Glu Ala
290 295 300

Leu Arg Ala Ala Ala Pro Val Gly Ala Val His Ile Ala Gly Pro Gly
305 310 315 320

Arg Gly Ala Arg Leu Val Ala Glu Glu Cys Arg
325 330

<210> 94

<211> 312

<212> PRT

<213> Borrelia burgdorferi

<400> 94

Met Lys Ile Lys Cys Lys Val His Ala Ser Leu Ala Leu Ile Lys Tyr
1 5 10 15

Trp Gly Lys Lys Asp Val Phe Leu Asn Ile Pro Ala Thr Ser Ser Leu

20 25 30

Ala Val Ser Val Asp Lys Phe Tyr Ser Ile Ser Glu Leu Glu Leu Ser
35 40 45

Asn Arg Asp Glu Ile Ile Leu Asn Ser Lys Pro Val Ile Leu Lys Asn
50 55 60

Arg Glu Lys Val Phe Phe Asp Tyr Ala Arg Lys Ile Leu Asn Glu Pro
65 70 75 80

Asn Val Arg Phe Lys Ile Lys Ser Lys Asn Asn Phe Pro Thr Ala Ala
85 90 95

Gly Leu Ala Ser Ser Ser Ser Gly Phe Ala Ser Ile Ala Ala Cys Ile
100 105 110

Leu Lys Tyr Phe Asn Lys Tyr Ser Cys Asn Ser Ala Ser Asn Leu Ala
115 120 125

Arg Val Gly Ser Ala Ser Ala Ala Arg Ala Ile Tyr Gly Gly Phe Thr
130 135 140

Ile Leu Lys Glu Gly Ser Lys Glu Ser Phe Gln Leu Arg Asp Gln Ser
145 150 155 160

Tyr Phe Asn Asp Leu Arg Ile Ile Phe Ala Ile Ile Asp Ser Asn Glu
165 170 175

Lys Glu Leu Ser Ser Arg Ala Ala Met Asn Ile Cys Lys Arg His Lys
180 185 190

Phe Tyr Tyr Asp Ala Trp Ile Ala Ser Ser Lys Lys Ile Phe Lys Asp
195 200 205

Ala Leu Tyr Phe Phe Leu Lys Lys Asp Phe Ile His Phe Gly Ala Thr
210 215 220

Ile Val Lys Ser Tyr Gln Asn Met Phe Ala Leu Met Phe Ala Ser Ser
225 230 235 240

Ile Phe Tyr Phe Lys Asn Ser Thr Ile Asp Leu Ile Arg Tyr Ala Ala
245 250 255

Asp Leu Arg Asn Glu Gly Ile Phe Val Phe Glu Thr Met Asp Ala Gly
260 265 270

Pro Gln Val Lys Phe Leu Cys Leu Glu Glu Asn Leu Asn Thr Ile Leu
275 280 285

Lys Gly Leu Lys Gln Asn Phe Thr Gly Ile Asp Phe Ile Val Ser Lys
290 295 300

Val Gly Cys Asp Leu Glu Trp Ile
305 310

<210> 95

<211> 292

<212> PRT

<213> Streptococcus pneumoniae

<400> 95

Met Thr Lys Lys Val Gly Val Gly Gln Ala His Ser Lys Ile Ile Leu
1 5 10 15

Ile Gly Glu His Ala Val Val Tyr Gly Tyr Pro Ala Ile Ser Leu Pro
20 25 30

Leu Leu Glu Val Glu Val Thr Cys Lys Val Val Ser Ala Glu Ser Pro
35 40 45

Trp Arg Leu Tyr Glu Glu Asp Thr Leu Ser Met Ala Val Tyr Ala Ser
50 55 60

Leu Glu Tyr Leu Asp Ile Thr Glu Ala Cys Val Arg Cys Glu Ile Asp
65 70 75 80

Ser Ala Ile Pro Glu Lys Arg Gly Met Gly Ser Ser Ala Ala Ile Ser
85 90 95

Ile Ala Ala Ile Arg Ala Val Phe Asp Tyr Tyr Gln Ala Asp Leu Pro
100 105 110

His Asp Val Leu Glu Ile Leu Val Asn Arg Ala Glu Met Ile Ala His
115 120 125

Met Asn Pro Ser Gly Leu Asp Ala Lys Thr Cys Leu Ser Asp Gln Pro
130 135 140

Ile Arg Phe Ile Lys Asn Val Gly Phe Thr Glu Leu Glu Met Asp Leu
145 150 155 160

Ser Ala Tyr Leu Val Ile Ala Asp Thr Gly Val Tyr Gly His Thr Arg
165 170 175

Glu Ala Ile Gln Val Val Gln Asn Lys Gly Lys Asp Ala Leu Pro Phe
180 185 190

Leu His Ala Leu Gly Glu Leu Thr Gln Gln Ala Glu Val Ala Ile Ser
195 200 205

Gln Lys Tyr Ala Glu Gly Leu Gly Leu Ile Phe Ser Gln Ala His Leu
210 215 220

His Leu Lys Glu Ile Gly Val Ser Ser Pro Glu Ala Asp Phe Leu Val
225 230 235 240

Glu Thr Ala Leu Ser Tyr Gly Ala Leu Gly Ala Lys Met Ser Gly Gly

245 250 255

Gly Leu Gly Gly Cys Ile Ile Ala Leu Val Thr Asn Leu Thr His Ala
260 265 270

Gln Glu Leu Ala Glu Arg Leu Glu Glu Lys Gly Ala Val Gln Thr Trp
275 280 285

Ile Glu Ser Leu
290

<210> 96

<211> 292

<212> PRT

<213> Streptococcus pyrogenes

<400> 96

Met Asn Glu Asn Ile Gly Tyr Gly Lys Ala His Ser Lys Ile Ile Leu
1 5 10 15

Ile Gly Glu His Ala Val Val Tyr Gly Tyr Pro Ala Ile Ala Leu Pro
20 25 30

Leu Thr Asp Ile Glu Val Val Cys His Ile Phe Pro Ala Asp Lys Pro
35 40 45

Leu Val Phe Asp Phe Tyr Asp Thr Leu Ser Thr Ala Ile Tyr Ala Ala
50 55 60

Leu Asp Tyr Leu Gln Arg Leu Gln Glu Pro Ile Ala Tyr Glu Ile Val
65 70 75 80

Ser Gln Val Pro Gln Lys Arg Gly Met Gly Ser Ser Ala Ala Val Ser
85 90 95

Ile Ala Ala Ile Arg Ala Val Phe Ser Tyr Cys Gln Glu Pro Leu Ser
100 105 110

Asp Asp Leu Leu Glu Ile Leu Val Asn Lys Ala Glu Ile Ile Ala His
115 120 125

Thr Asn Pro Ser Gly Leu Asp Ala Lys Thr Cys Leu Ser Asp His Ala
130 135 140

Ile Lys Phe Ile Arg Asn Ile Gly Phe Glu Thr Ile Glu Ile Ala Leu
145 150 155 160

Asn Gly Tyr Leu Ile Ile Ala Asp Thr Gly Ile His Gly His Thr Arg
165 170 175

Glu Ala Val Asn Lys Val Ala Gln Phe Glu Glu Thr Asn Leu Pro Tyr

180 185 190

Leu Ala Lys Leu Gly Ala Leu Thr Gln Ala Leu Glu Arg Ala Ile Asn
195 200 205

Gln Lys Asn Lys Val Ala Ile Gly Gln Leu Met Thr Gln Ala His Ser
210 215 220

Ala Leu Lys Ala Ile Gly Val Ser Ile Ser Lys Ala Asp Gln Leu Val
225 230 235 240

Glu Ala Ala Leu Arg Ala Gly Ala Leu Gly Ala Lys Met Thr Gly Gly
245 250 255

Gly Leu Gly Gly Cys Met Ile Ala Leu Ala Asp Thr Lys Asp Met Ala
260 265 270

Glu Lys Ile Ser His Lys Leu Lys Glu Glu Gly Ala Val Asn Thr Trp
275 280 285

Ile Gln Met Leu
290

<210> 97

<211> 314

<212> PRT

<213> Enterococcus faecalis

<400> 97

Met Asn Ile Lys Lys Gln Gly Leu Gly Gln Ala Thr Gly Lys Ile Ile
1 5 10 15

Leu Met Gly Glu His Ala Val Val Tyr Gly Glu Pro Ala Ile Ala Phe
20 25 30

Pro Phe Gln Ala Thr Glu Ile Thr Ala Val Phe Thr Pro Ala Lys Thr
35 40 45

Met Gln Ile Asp Cys Ala Tyr Phe Thr Gly Leu Leu Glu Asp Val Pro
50 55 60

Gln Glu Leu Ala Asn Ile Lys Glu Val Val Gln Gln Thr Leu His Phe
65 70 75 80

Leu Lys Glu Asp Thr Phe Lys Gly Thr Leu Thr Leu Thr Ser Thr Ile
85 90 95

Pro Ala Glu Arg Gly Met Gly Ser Ser Ala Ala Thr Ala Val Ala Ile
100 105 110

Val Arg Ser Leu Phe Asp Tyr Phe Asp Tyr Ala Tyr Thr Tyr Gln Glu

115 120 125

Leu Phe Glu Leu Val Ser Leu Ser Glu Lys Ile Ala His Gly Asn Pro
130 135 140

Ser Gly Ile Asp Ala Ala Ala Thr Ser Gly Ala Asp Pro Leu Phe Phe
145 150 155 160

Thr Arg Gly Phe Pro Pro Thr His Phe Ser Met Asn Leu Ser Asn Ala
165 170 175

Tyr Leu Val Val Ala Asp Thr Gly Ile Lys Gly Gln Thr Arg Glu Ala
180 185 190

Val Lys Asp Ile Ala Gln Leu Ala Gln Asn Asn Pro Thr Ala Ile Ala
195 200 205

Glu Thr Met Lys Gln Leu Gly Ser Phe Thr Lys Glu Ala Lys Gln Ala
210 215 220

Ile Leu Gln Asp Asp Lys Gln Lys Leu Gly Gln Leu Met Thr Leu Ala
225 230 235 240

Gln Glu Gln Leu Gln Gln Leu Thr Val Ser Asn Asp Met Leu Asp Arg
245 250 255

Leu Val Ala Leu Ser Leu Glu His Gly Ala Leu Gly Ala Lys Leu Thr
260 265 270

Gly Gly Gly Arg Gly Gly Cys Met Ile Ala Leu Thr Asp Asn Lys Lys
275 280 285

Thr Ala Gln Thr Ile Ala Gln Thr Leu Glu Glu Asn Gly Ala Val Ala
290 295 300

Thr Trp Ile Gln Ser Leu Glu Val Lys Lys
305 310

<210> 98

<211> 314

<212> PRT

<213> Enterococcus faecium

<400> 98

Met Ala Asn Tyr Gly Gln Gly Glu Ser Ser Gly Lys Ile Ile Leu Met
1 5 10 15

Gly Glu His Ala Val Val Tyr Gly Glu Pro Ala Ile Ala Phe Pro Phe
20 25 30

Tyr Ala Thr Lys Val Thr Ala Phe Leu Glu Glu Leu Asp Ala Met Asp

35 40 45

Asp Gln Leu Val Ser Ser Tyr Tyr Ser Gly Asn Leu Ala Glu Ala Pro
50 55 60

His Ala Leu Lys Asn Ile Lys Lys Leu Phe Ile His Leu Lys Lys Gln
65 70 75 80

His Asp Ile Gln Lys Asn Leu Gln Leu Thr Ile Glu Ser Thr Ile Pro
85 90 95

Ala Glu Arg Gly Met Gly Ser Ser Ala Ala Val Ala Thr Ala Val Thr
100 105 110

Arg Ala Phe Tyr Asp Tyr Leu Ala Phe Pro Leu Ser Arg Glu Ile Leu
115 120 125

Leu Glu Asn Val Gln Leu Ser Glu Lys Ile Ala His Gly Asn Pro Ser
130 135 140

Gly Ile Asp Ala Ala Ala Thr Ser Ser Leu Gln Pro Ile Tyr Phe Thr
145 150 155 160

Lys Gly His Pro Phe Asp Tyr Phe Ser Leu Asn Ile Asp Ala Phe Leu
165 170 175

Ile Val Ala Asp Thr Gly Ile Lys Gly Gln Thr Arg Glu Ala Val Lys
180 185 190

Asp Val Ala His Leu Phe Glu Thr Gln Pro His Glu Thr Gly Gln Met
195 200 205

Ile Gln Lys Leu Gly Tyr Leu Thr Lys Gln Ala Lys Gln Ala Ile Ile
210 215 220

Glu Asn Ser Pro Glu Thr Leu Ala Gln Thr Met Asp Glu Ser Gln Ser
225 230 235 240

Leu Leu Glu Lys Leu Thr Ile Ser Asn Asp Phe Leu Asn Leu Leu Ile
245 250 255

Gln Thr Ala Lys Asp Thr Gly Ala Leu Gly Ala Lys Leu Thr Gly Gly
260 265 270

Gly Arg Gly Gly Cys Met Ile Ala Leu Ala Gln Thr Lys Thr Lys Ala
275 280 285

Gln Glu Ile Ser Gln Ala Leu Glu Asp Ala Gly Ala Ala Glu Thr Trp
290 295 300

Ile Gln Gly Leu Gly Val His Thr Tyr Val
305 310

<210> 99

<211> 307

<212> PRT

<213> Staphylococcus haemolyticus

<400> 99

Met Val Gln Arg Gly Tyr Gly Glu Ser Asn Gly Lys Ile Ile Leu Ile
1 5 10 15

Gly Glu His Ala Val Thr Phe Gly Glu Pro Ala Ile Ala Ile Pro Phe
20 25 30

Thr Ser Gly Lys Val Lys Val Leu Ile Glu Ser Leu Glu Lys Gly Asn
35 40 45

Tyr Ser Ala Ile Gln Ser Asp Val Tyr Asp Gly Pro Leu Tyr Asp Ala
50 55 60

Pro Glu His Leu Lys Ser Leu Ile Gly His Phe Val Glu Asn Lys Lys
65 70 75 80

Val Glu Glu Pro Leu Leu Ile Lys Ile Gln Ala Asn Leu Pro Pro Ser
85 90 95

Arg Gly Leu Gly Ser Ser Ala Ala Val Ala Val Ala Phe Ile Arg Ala
100 105 110

Ser Tyr Asp Tyr Leu Gly Leu Pro Leu Thr Asp Lys Glu Leu Leu Glu
115 120 125

Asn Ala Asp Trp Ala Glu Arg Ile Ala His Gly Lys Pro Ser Gly Ile
130 135 140

Asp Thr Lys Thr Ile Val Thr Asn Gln Pro Val Trp Tyr Gln Lys Gly
145 150 155 160

Glu Val Glu Ile Leu Lys Thr Leu Asp Leu Asp Gly Tyr Met Val Val
165 170 175

Ile Asp Thr Gly Val Lys Gly Ser Thr Lys Gln Ala Val Glu Asp Val
180 185 190

His Gln Leu Cys Asp Asn Asp Lys Asn Tyr Met Gln Val Val Lys His
195 200 205

Ile Gly Ser Leu Val Tyr Ser Ala Ser Glu Ala Ile Glu His His Ser
210 215 220

Phe Asp Gln Leu Ala Thr Ile Phe Asn Gln Cys Gln Asp Asp Leu Arg
225 230 235 240

Thr Leu Thr Val Ser His Asp Lys Ile Glu Met Phe Leu Arg Leu Gly
245 250 255

Glu Glu Asn Gly Ser Val Ala Gly Lys Leu Thr Gly Gly Gly Arg Gly

260 265 270

Gly Ser Met Leu Ile Leu Ala Lys Glu Leu Gln Thr Ala Lys Asn Ile
275 280 285

Val Ala Ala Val Glu Lys Ala Gly Ala Gln His Thr Trp Ile Glu Lys
290 295 300

Leu Gly Gly
305

<210> 100

<211> 306

<212> PRT

<213> Staphylococcus epidermis

<400> 100

Met Thr Arg Gln Gly Tyr Gly Glu Ser Thr Gly Lys Ile Ile Leu Met
1 5 10 15

Gly Glu His Ala Val Thr Phe Gly Gln Pro Ala Ile Ala Ile Pro Phe
20 25 30

Asn Ala Gly Lys Ile Lys Val Leu Ile Glu Ser Leu Asp Glu Gly Asn
35 40 45

Tyr Ser Ser Ile Thr Ser Asp Val Tyr Asp Gly Met Leu Tyr Asp Ala
50 55 60

Pro Glu His Leu Lys Ser Ile Ile Asn Arg Phe Val Glu Lys Ser Gly
65 70 75 80

Val Lys Glu Pro Leu Ser Val Lys Ile Gln Thr Asn Leu Pro Pro Ser
85 90 95

Arg Gly Leu Gly Ser Ser Ala Ala Val Ala Val Ala Phe Val Arg Ala
100 105 110

Ser Tyr Asp Phe Met Asp Gln Pro Leu Asp Asp Lys Thr Leu Ile Lys
115 120 125

Glu Ala Asn Trp Ala Glu Gln Ile Ala His Gly Lys Pro Ser Gly Ile
130 135 140

Asp Thr Gln Thr Ile Val Ser Asn Lys Pro Val Trp Phe Lys Gln Gly
145 150 155 160

Gln Ala Glu Thr Leu Lys Ser Leu Lys Leu Asn Gly Tyr Met Val Val
165 170 175

Ile Asp Thr Gly Val Lys Gly Ser Thr Lys Gln Ala Val Glu Asp Val

180 185 190

His Val Leu Cys Glu Ser Asp Glu Tyr Met Lys Tyr Ile Glu His Ile
195 200 205

Gly Thr Leu Val His Ser Ala Ser Glu Ser Ile Glu Gln His Asp Phe
210 215 220

His His Leu Ala Asp Ile Phe Asn Ala Cys Gln Glu Asp Leu Arg His
225 230 235 240

Leu Thr Val Ser His Asp Lys Ile Glu Lys Leu Leu Gln Ile Gly Lys
245 250 255

Glu His Gly Ala Ile Ala Gly Lys Leu Thr Gly Gly Gly Arg Gly Gly
260 265 270

Ser Met Leu Leu Leu Ala Glu Asn Leu Lys Thr Ala Lys Thr Ile Val
275 280 285

Ala Ala Val Glu Lys Ala Gly Ala Ala His Thr Trp Ile Glu His Leu
290 295 300

Gly Gly
305

<210> 101

<211> 306

<212> PRT

<213> Staphylococcus aureus

<400> 101

Met Thr Arg Lys Gly Tyr Gly Glu Ser Thr Gly Lys Ile Ile Leu Ile
1 5 10 15

Gly Glu His Ala Val Thr Phe Gly Glu Pro Ala Ile Ala Val Pro Phe
20 25 30

Asn Ala Gly Lys Ile Lys Val Leu Ile Glu Ala Leu Glu Ser Gly Asn
35 40 45

Tyr Ser Ser Ile Lys Ser Asp Val Tyr Asp Gly Met Leu Tyr Asp Ala
50 55 60

Pro Asp His Leu Lys Ser Leu Val Asn Arg Phe Val Glu Leu Asn Asn
65 70 75 80

Ile Thr Glu Pro Leu Ala Val Thr Ile Gln Thr Asn Leu Pro Pro Ser
85 90 95

Arg Gly Leu Gly Ser Ser Ala Ala Val Ala Val Ala Phe Val Arg Ala

100 105 110

Ser Tyr Asp Phe Leu Gly Lys Ser Leu Thr Lys Glu Glu Leu Ile Glu
115 120 125

Lys Ala Asn Trp Ala Glu Gln Ile Ala His Gly Lys Pro Ser Gly Ile
130 135 140

Asp Thr Gln Thr Ile Val Ser Gly Lys Pro Val Trp Phe Gln Lys Gly
145 150 155 160

Gln Ala Glu Thr Leu Lys Thr Leu Ser Leu Asp Gly Tyr Met Val Val
165 170 175

Ile Asp Thr Gly Val Lys Gly Ser Thr Arg Gln Ala Val Glu Asp Val
180 185 190

His Lys Leu Cys Glu Asp Pro Gln Tyr Met Ser His Val Lys His Ile
195 200 205

Gly Lys Leu Val Leu Arg Ala Ser Asp Val Ile Glu His His Asn Phe
210 215 220

Glu Ala Leu Ala Asp Ile Phe Asn Glu Cys His Ala Asp Leu Lys Ala
225 230 235 240

Leu Thr Val Ser His Asp Lys Ile Glu Gln Leu Met Lys Ile Gly Lys
245 250 255

Glu Asn Gly Ala Ile Ala Gly Lys Leu Thr Gly Ala Gly Arg Gly Gly
260 265 270

Ser Met Leu Leu Leu Ala Lys Asp Leu Pro Thr Ala Lys Asn Ile Val
275 280 285

Lys Ala Val Glu Lys Ala Gly Ala Ala His Thr Trp Ile Glu Asn Leu
290 295 300

Gly Gly
305

<210> 102

<211> 345

<212> PRT

<213> Streptomyces sp. CL190

<400> 102

Met Gln Lys Arg Gln Arg Glu Leu Ser Ala Leu Thr Leu Pro Thr Ser
1 5 10 15

Ala Glu Gly Val Ser Glu Ser His Arg Ala Arg Ser Val Gly Ile Gly

20 25 30

Arg Ala His Ala Lys Ala Ile Leu Leu Gly Glu His Ala Val Val Tyr
35 40 45

Gly Ala Pro Ala Leu Ala Leu Pro Ile Pro Gln Leu Thr Val Thr Ala
50 55 60

Ser Val Gly Trp Ser Ser Glu Ala Ser Asp Ser Ala Gly Gly Leu Ser
65 70 75 80

Tyr Thr Met Thr Gly Thr Pro Ser Arg Ala Leu Val Thr Gln Ala Ser
85 90 95

Asp Gly Leu His Arg Leu Thr Ala Glu Phe Met Ala Arg Met Gly Val
100 105 110

Thr Asn Ala Pro His Leu Asp Val Ile Leu Asp Gly Ala Ile Pro His
115 120 125

Gly Arg Gly Leu Gly Ser Ser Ala Ala Gly Ser Arg Ala Ile Ala Leu
130 135 140

Ala Leu Ala Asp Leu Phe Gly His Glu Leu Ala Glu His Thr Ala Tyr
145 150 155 160

Glu Leu Val Gln Thr Ala Glu Asn Met Ala His Gly Arg Ala Ser Gly
165 170 175

Val Asp Ala Met Thr Val Gly Ala Ser Arg Pro Leu Leu Phe Gln Gln
180 185 190

Gly Arg Thr Glu Arg Leu Ala Ile Gly Cys Asp Ser Leu Phe Ile Val
195 200 205

Ala Asp Ser Gly Val Pro Gly Ser Thr Lys Glu Ala Val Glu Met Leu
210 215 220

Arg Glu Gly Phe Thr Arg Ser Ala Gly Thr Gln Glu Arg Phe Val Gly
225 230 235 240

Arg Ala Thr Glu Leu Thr Glu Ala Ala Arg Gln Ala Leu Ala Asp Gly
245 250 255

Arg Pro Glu Glu Leu Gly Ser Gln Leu Thr Tyr Tyr His Glu Leu Leu
260 265 270

His Glu Ala Arg Leu Ser Thr Asp Gly Ile Asp Ala Leu Val Glu Ala
275 280 285

Ala Leu Lys Ala Gly Ser Leu Gly Ala Lys Ile Thr Gly Gly Gly Leu
290 295 300

Gly Gly Cys Met Ile Ala Gln Ala Arg Pro Glu Gln Ala Arg Glu Val
305 310 315 320

Thr Arg Gln Leu His Glu Ala Gly Ala Val Gln Thr Trp Val Val Pro

325 330 335

Leu Lys Gly Leu Asp Asn His Ala Gln
340 345

<210> 103

<211> 334

<212> PRT

<213> Streptomyces griseolosporeus

<400> 103

Met Thr Leu Pro Thr Ser Val Glu Glu Gly Ser Lys Ala His Arg Ala
1 5 10 15

Arg Ala Val Gly Thr Gly Arg Ala His Ala Lys Ala Ile Leu Leu Gly
20 25 30

Glu His Ala Val Val Tyr Gly Thr Pro Ala Leu Ala Met Pro Ile Pro
35 40 45

Gln Leu Ala Val Thr Ala Ser Ala Gly Trp Ser Gly Arg Ser Ala Glu
50 55 60

Ser Arg Gly Gly Pro Thr Phe Thr Met Thr Gly Ser Ala Ser Arg Ala
65 70 75 80

Val Thr Ala Gln Ala Leu Asp Gly Leu Arg Arg Leu Thr Ala Ser Val
85 90 95

Lys Ala His Thr Gly Val Thr Asp Gly Gln His Leu Asp Val Ser Leu
100 105 110

Asp Gly Ala Ile Pro Pro Gly Arg Gly Leu Gly Ser Ser Ala Ala Asn
115 120 125

Ala Arg Ala Ile Ile Leu Ala Leu Ala Asp Leu Phe Gly Arg Glu Leu
130 135 140

Thr Glu Gly Glu Val Phe Asp Leu Val Gln Glu Ala Glu Asn Leu Thr
145 150 155 160

His Gly Arg Ala Ser Gly Val Asp Ala Val Thr Val Gly Ala Thr Ala
165 170 175

Pro Leu Leu Phe Arg Ala Gly Thr Ala Gln Ala Leu Asp Ile Gly Cys
180 185 190

Asp Ala Leu Phe Val Val Ala Asp Ser Gly Thr Ala Gly Ser Thr Lys
195 200 205

Glu Ala Ile Glu Leu Leu Arg Ala Gly Phe Arg Ala Gly Ala Gly Lys

210 215 220

Glu Glu Arg Phe Met His Arg Ala Ala His Leu Val Asp Asp Ala Arg
225 230 235 240

Ala Ser Leu Ala Glu Gly Glu Pro Glu Ala Phe Gly Ser Cys Leu Thr
245 250 255

Glu Tyr His Gly Leu Leu Arg Gly Ala Gly Leu Ser Thr Asp Arg Ile
260 265 270

Asp Ala Leu Val Asp Ala Ala Leu Gln Ala Asp Ser Leu Gly Ala Lys
275 280 285

Ile Thr Gly Gly Gly Leu Gly Gly Cys Val Leu Ala Met Ser Arg Pro
290 295 300

Glu Arg Ala Glu Glu Val Ala Arg Gln Leu His Ala Ala Gly Ala Val
305 310 315 320

Arg Thr Trp Ala Val Gln Leu Arg Arg Ser Thr His Glu Arg
325 330

<210> 104

<211> 296

<212> PRT

<213> Borrelia burgdorferi

<400> 104

Met Leu Arg Ile Arg Lys Pro Ala Lys Ile Leu Phe Leu Gly Glu His
1 5 10 15

Ser Ala Val Tyr Gly Phe Pro Val Ile Gly Ala Thr Val Pro Ile Tyr
20 25 30

Met Asp Leu Ile Tyr Ser Val Ser Lys Asn Trp Lys Tyr Leu Gly Lys
35 40 45

Pro Ser Thr Arg Leu Asn Ser Leu Ile Ser Phe Ile Val Ser Asn Tyr
50 55 60

Ser Lys Val Asn Pro Ile Glu Phe Asp Ile Ile Ser Glu Ile Pro Ile
65 70 75 80

Gly Val Gly Leu Gly Ser Ser Ala Ser Leu Ser Leu Cys Phe Ala Glu
85 90 95

Tyr Ile Thr Ser His Phe Glu Tyr Lys Asp Cys Asn Lys Ile Leu Leu
100 105 110

Ala Asn Gln Ile Glu Asn Ile Phe His Gly Lys Ser Ser Gly Met Asp

115 120 125

Ile Arg Leu Ile Asp Leu Asn Gly Thr Phe Tyr Leu Glu Lys Lys Glu
130 135 140

Asn Val Leu His Ser Lys Lys Ile Lys Asp Ser Gly Phe Tyr Phe Leu
145 150 155 160

Ile Gly Ala Ile Lys Arg Asp Leu Thr Thr Lys Glu Ile Val Val Asn
165 170 175

Leu Lys Lys Asp Leu Leu Ser Asn Ala Tyr Leu Phe Val Phe Ile Glu
180 185 190

Lys Leu Gly Leu Ala Val Ser Asn Ser Tyr Ala Ser Phe Gln Asn Lys
195 200 205

Asp Val Tyr Ser Leu Ala Asn Glu Met Asn Ile Ala Gln Cys Cys Leu
210 215 220

Lys Arg Leu Gly Leu Ser Asn Asp Thr Leu Asp Trp Leu Ile Ser Glu
225 230 235 240

Gly Ile Lys Leu Gly Ala Leu Ser Gly Lys Leu Ser Gly Ala Gly Lys
245 250 255

Gly Gly Ala Phe Ile Phe Leu Phe Glu Ser Leu Ile Lys Ala Asn Ile
260 265 270

Val Gln Lys Glu Leu Asn Asn Met Leu Asp Ser Lys Ile Asp Leu Leu
275 280 285

Leu Lys Leu Lys Val Ile Glu Thr
290 295

<210> 105

<211> 336

<212> PRT

<213> Streptococcus pneumoniae

<400> 105

Met Ile Ala Val Lys Thr Cys Gly Lys Leu Tyr Trp Ala Gly Glu Tyr
1 5 10 15

Ala Ile Leu Glu Pro Gly Gln Leu Ala Leu Ile Lys Asp Ile Pro Ile
20 25 30

Tyr Met Arg Ala Glu Ile Ala Phe Ser Asp Ser Tyr Arg Ile Tyr Ser
35 40 45

Asp Met Phe Asp Phe Ala Val Asp Leu Arg Pro Asn Pro Asp Tyr Ser

50 55 60

Leu Ile Gln Glu Thr Ile Ala Leu Met Gly Asp Phe Leu Ala Val Arg
65 70 75 80

Gly Gln Asn Leu Arg Pro Phe Ser Leu Lys Ile Cys Gly Lys Met Glu
85 90 95

Arg Glu Gly Lys Lys Phe Gly Leu Gly Ser Ser Gly Ser Val Val Val
100 105 110

Leu Val Val Lys Ala Leu Leu Ala Leu Tyr Asn Leu Ser Val Asp Gln
115 120 125

Asn Leu Leu Phe Lys Leu Thr Ser Ala Val Leu Leu Lys Arg Gly Asp
130 135 140

Asn Gly Ser Met Gly Asp Leu Ala Cys Ile Val Ala Glu Asp Leu Val
145 150 155 160

Leu Tyr Gln Ser Phe Asp Arg Gln Lys Ala Ala Ala Trp Leu Glu Glu
165 170 175

Glu Asn Leu Ala Thr Val Leu Glu Arg Asp Trp Gly Phe Phe Ile Ser
180 185 190

Gln Val Lys Pro Thr Leu Glu Cys Asp Phe Leu Val Gly Trp Thr Lys
195 200 205

Glu Val Ala Val Ser Ser His Met Val Gln Gln Ile Lys Gln Asn Ile
210 215 220

Asn Gln Asn Phe Leu Ser Ser Ser Lys Glu Thr Val Val Ser Leu Val
225 230 235 240

Glu Ala Leu Glu Gln Gly Lys Ala Glu Lys Val Ile Glu Gln Val Glu
245 250 255

Val Ala Ser Lys Leu Leu Glu Gly Leu Ser Thr Asp Ile Tyr Thr Pro
260 265 270

Leu Leu Arg Gln Leu Lys Glu Ala Ser Gln Asp Leu Gln Ala Val Ala
275 280 285

Lys Ser Ser Gly Ala Gly Gly Gly Asp Cys Gly Ile Ala Leu Ser Phe
290 295 300

Asp Ala Gln Ser Ser Arg Asn Thr Leu Lys Asn Arg Trp Ala Asp Leu
305 310 315 320

Gly Ile Glu Leu Leu Tyr Gln Glu Arg Ile Gly His Asp Asp Lys Ser
325 330 335

<210> 106

<211> 335

<212> PRT

<213> Streptococcus pyrogenes

<400> 106

Met Ser Asn Tyr Cys Val Gln Thr Gly Gly Lys Leu Tyr Leu Thr Gly
1 5 10 15

Glu Tyr Ala Ile Leu Ile Pro Gly Gln Lys Ala Leu Ile His Phe Ile
20 25 30

Pro Leu Met Met Thr Ala Glu Ile Ser Pro Ala Ala His Ile Gln Leu
35 40 45

Ala Ser Asp Met Phe Ser His Lys Ala Gly Met Thr Pro Asp Ala Ser
50 55 60

Tyr Ala Leu Ile Gln Ala Thr Val Lys Thr Phe Ala Asp Tyr Leu Gly
65 70 75 80

Gln Ser Ile Asp Gln Leu Glu Pro Phe Ser Leu Ile Ile Thr Gly Lys
85 90 95

Met Glu Arg Asp Gly Lys Lys Phe Gly Ile Gly Ser Ser Gly Ser Val
100 105 110

Thr Leu Leu Thr Leu Lys Ala Leu Ser Ala Tyr Tyr Gln Ile Thr Leu
115 120 125

Thr Pro Glu Leu Leu Phe Lys Leu Ala Ala Tyr Thr Leu Leu Lys Gln
130 135 140

Gly Asp Asn Gly Ser Met Gly Asp Ile Ala Cys Ile Ala Tyr Gln Thr
145 150 155 160

Leu Val Ala Tyr Thr Ser Phe Asp Arg Glu Gln Val Ser Asn Trp Leu
165 170 175

Gln Thr Met Pro Leu Lys Lys Leu Leu Val Lys Asp Trp Gly Tyr His
180 185 190

Ile Gln Val Ile Gln Pro Ala Leu Pro Cys Asp Phe Leu Val Gly Trp
195 200 205

Thr Lys Ile Pro Ala Ile Ser Arg Gln Met Ile Gln Gln Val Thr Ala
210 215 220

Ser Ile Thr Pro Ala Phe Leu Arg Thr Ser Tyr Gln Leu Thr Gln Ser
225 230 235 240

Ala Met Val Ala Leu Gln Glu Gly His Lys Glu Glu Leu Lys Lys Ser
245 250 255

Leu Ala Gly Ala Ser His Leu Leu Lys Glu Leu His Pro Ala Ile Tyr

260 265 270

His Pro Lys Leu Val Thr Leu Val Ala Ala Cys Gln Lys Gln Asp Ala
275 280 285

Val Ala Lys Ser Ser Gly Ala Gly Gly Gly Asp Cys Gly Ile Ala Leu
290 295 300

Ala Phe Asn Gln Asp Ala Arg Asp Thr Leu Ile Ser Lys Trp Gln Glu
305 310 315 320

Ala Asp Ile Ala Leu Leu Tyr Gln Glu Arg Trp Gly Glu Asn Asp
325 330 335

<210> 107

<211> 368

<212> PRT

<213> Enterococcus faecalis

<400> 107

Met Ile Glu Val Thr Thr Pro Gly Lys Leu Phe Ile Ala Gly Glu Tyr
1 5 10 15

Ala Val Val Glu Pro Gly His Pro Ala Ile Ile Val Ala Val Asp Gln
20 25 30

Phe Val Thr Val Thr Val Glu Glu Thr Thr Asp Glu Gly Ser Ile Gln
35 40 45

Ser Ala Gln Tyr Ser Ser Leu Pro Ile Arg Trp Thr Arg Arg Asn Gly
50 55 60

Glu Leu Val Leu Asp Ile Arg Glu Asn Pro Phe His Tyr Val Leu Ala
65 70 75 80

Ala Ile His Leu Thr Glu Lys Tyr Ala Gln Glu Gln Asn Lys Glu Leu
85 90 95

Ser Phe Tyr His Leu Lys Val Thr Ser Glu Leu Asp Ser Ser Asn Gly
100 105 110

Arg Lys Tyr Gly Leu Gly Ser Ser Gly Ala Val Thr Val Gly Thr Val
115 120 125

Lys Ala Leu Asn Ile Phe Tyr Asp Leu Gly Leu Glu Asn Glu Glu Ile
130 135 140

Phe Lys Leu Ser Ala Leu Ala His Leu Ala Val Gln Gly Asn Gly Ser
145 150 155 160

Cys Gly Asp Ile Ala Ala Ser Cys Tyr Gly Gly Trp Ile Ala Phe Ser

165 170 175

Thr Phe Asp His Asp Trp Val Asn Gln Lys Val Thr Thr Glu Thr Leu
180 185 190

Thr Asp Leu Leu Ala Met Asp Trp Pro Glu Leu Met Ile Phe Pro Leu
195 200 205

Lys Val Pro Lys Gln Leu Arg Leu Leu Ile Gly Trp Thr Gly Ser Pro
210 215 220

Ala Ser Thr Ser Asp Leu Val Asp Arg Val His Gln Ser Lys Glu Glu
225 230 235 240

Lys Gln Ala Ala Tyr Glu Gln Phe Leu Met Lys Ser Arg Leu Cys Val
245 250 255

Glu Thr Met Ile Asn Gly Phe Asn Thr Gly Lys Ile Ser Val Ile Gln
260 265 270

Lys Gln Ile Thr Lys Asn Arg Gln Leu Leu Ala Glu Leu Ser Ser Leu
275 280 285

Thr Gly Val Val Ile Glu Thr Glu Ala Leu Lys Asn Leu Cys Asp Leu
290 295 300

Ala Glu Ser Tyr Thr Gly Ala Ala Lys Ser Ser Gly Ala Gly Gly Gly
305 310 315 320

Asp Cys Gly Ile Val Ile Phe Arg Gln Lys Ser Gly Ile Leu Pro Leu
325 330 335

Met Thr Ala Trp Glu Lys Asp Gly Ile Thr Pro Leu Pro Leu His Val
340 345 350

Tyr Thr Tyr Gly Gln Lys Glu Cys Lys Glu Lys His Glu Ser Lys Arg
355 360 365

<210> 108

<211> 361

<212> PRT

<213> Enterococcus faecium

<400> 108

Met Ile Glu Val Ser Ala Pro Gly Lys Leu Tyr Ile Ala Gly Glu Tyr
1 5 10 15

Ala Val Val Glu Thr Gly His Pro Ala Val Ile Ala Ala Val Asp Gln
20 25 30

Phe Val Thr Val Thr Val Glu Ser Ala Arg Lys Val Gly Ser Ile Gln

35 40 45

Ser Ala Gln Tyr Ser Gly Met Pro Val Arg Trp Thr Arg Arg Asn Gly
50 55 60

Glu Leu Val Leu Asp Ile Arg Glu Asn Pro Phe His Tyr Ile Leu Ala
65 70 75 80

Ala Ile Arg Leu Thr Glu Lys Tyr Ala Gln Glu Lys Asn Ile Leu Leu
85 90 95

Ser Phe Tyr Asp Leu Lys Val Thr Ser Glu Leu Asp Ser Ser Asn Gly
100 105 110

Arg Lys Tyr Gly Leu Gly Ser Ser Gly Ala Val Thr Val Ala Thr Val
115 120 125

Lys Ala Leu Asn Val Phe Tyr Ala Leu Asn Leu Ser Gln Leu Glu Ile
130 135 140

Phe Lys Ile Ala Ala Leu Ala Asn Leu Ala Val Gln Asp Asn Gly Ser
145 150 155 160

Cys Gly Asp Ile Ala Ala Ser Cys Tyr Gly Gly Trp Ile Ala Phe Ser
165 170 175

Thr Phe Asp His Pro Trp Leu Gln Glu Gln Glu Thr Gln His Ser Ile
180 185 190

Ser Glu Leu Leu Ala Leu Asp Trp Pro Gly Leu Ser Ile Glu Pro Leu
195 200 205

Ile Ala Pro Glu Asp Leu Arg Leu Leu Ile Gly Trp Thr Gly Ser Pro
210 215 220

Ala Ser Thr Ser Asp Leu Val Asp Gln Val His Arg Ser Arg Glu Asp
225 230 235 240

Lys Met Val Ala Tyr Gln Leu Phe Leu Lys Asn Ser Thr Glu Cys Val
245 250 255

Asn Glu Met Ile Lys Gly Phe Lys Glu Asn Asn Val Thr Leu Ile Gln
260 265 270

Gln Met Ile Arg Lys Asn Arg Gln Leu Leu His Asp Leu Ser Ala Ile
275 280 285

Thr Gly Val Val Ile Glu Thr Pro Ala Leu Asn Lys Leu Cys Asn Leu
290 295 300

Ala Glu Gln Tyr Glu Gly Ala Ala Lys Ser Ser Gly Ala Gly Gly Gly
305 310 315 320

Asp Cys Gly Ile Val Ile Val Asp Gln Lys Ser Gly Ile Leu Pro Leu
325 330 335

Met Ser Ala Trp Glu Lys Ala Glu Ile Thr Pro Leu Pro Leu His Val

340 345 350

Tyr Ser Asp Gln Arg Lys Glu Asn Arg
355 360

<210> 109

<211> 358

<212> PRT

<213> Staphylococcus haemolyticus

<400> 109

Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Val Ala Gly Glu Tyr
1 5 10 15

Ala Val Thr Glu Pro Gly Tyr Lys Ser Val Leu Ile Ala Val Asp Arg
20 25 30

Phe Val Thr Ala Ser Ile Glu Ala Ser Asn Ala Val Thr Ser Thr Ile
35 40 45

His Ser Lys Thr Leu His Tyr Glu Pro Val Thr Phe Asn Arg Asn Glu
50 55 60

Asp Lys Ile Asp Ile Ser Asp Ala Asn Ala Ala Ser Gln Leu Lys Tyr
65 70 75 80

Val Val Thr Ala Ile Glu Val Phe Glu Gln Tyr Ala Arg Ser Cys Asn
85 90 95

Val Lys Leu Lys His Phe His Leu Glu Ile Asp Ser Asn Leu Asp Asp
100 105 110

Ala Ser Gly Asn Lys Tyr Gly Leu Gly Ser Ser Ala Ala Val Leu Val
115 120 125

Ser Val Val Lys Ala Leu Asn Glu Phe Tyr Asp Met Gln Leu Ser Asn
130 135 140

Leu Tyr Ile Tyr Lys Leu Ala Val Ile Ser Asn Met Arg Leu Gln Ser
145 150 155 160

Leu Ser Ser Cys Gly Asp Ile Ala Val Ser Val Tyr Ser Gly Trp Leu
165 170 175

Ala Tyr Ser Thr Phe Asp His Asp Trp Val Lys Gln Gln Met Glu Glu
180 185 190

Thr Ser Val Asn Glu Val Leu Glu Lys Asn Trp Pro Gly Leu His Ile
195 200 205

Glu Pro Leu Gln Ala Pro Glu Asn Met Glu Val Leu Ile Gly Trp Thr

210 215 220

Gly Ser Pro Ala Ser Ser Pro His Leu Val Ser Glu Val Lys Arg Leu
225 230 235 240

Lys Ser Asp Pro Ser Phe Tyr Gly Arg Phe Leu Asp Gln Ser His Thr
245 250 255

Cys Val Glu Asn Leu Ile Tyr Ala Phe Lys Thr Asp Asn Ile Lys Gly
260 265 270

Val Gln Lys Met Ile Arg Gln Asn Arg Met Ile Ile Gln Gln Met Asp
275 280 285

Asn Glu Ala Thr Val Asp Ile Glu Thr Glu Asn Leu Lys Met Leu Cys
290 295 300

Asp Ile Gly Glu Arg Tyr Gly Ala Ala Ala Lys Thr Ser Gly Ala Gly
305 310 315 320

Gly Gly Asp Cys Gly Ile Ala Ile Ile Asp Asn Arg Ile Asp Lys Asn
325 330 335

Arg Ile Tyr Asn Glu Trp Ala Ser His Gly Ile Lys Pro Leu Lys Phe
340 345 350

Lys Ile Tyr His Gly Gln
355

<210> 110

<211> 358

<212> PRT

<213> Staphylococcus epidermis

<400> 110

Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Ile Ala Gly Glu Tyr
1 5 10 15

Ala Val Thr Glu Pro Gly Tyr Lys Ser Ile Leu Ile Ala Val Asn Arg
20 25 30

Phe Val Thr Ala Thr Ile Glu Ala Ser Asn Lys Val Glu Gly Ser Ile
35 40 45

His Ser Lys Thr Leu His Tyr Glu Pro Val Lys Phe Asp Arg Asn Glu
50 55 60

Asp Arg Ile Glu Ile Ser Asp Val Gln Ala Ala Lys Gln Leu Lys Tyr
65 70 75 80

Val Val Thr Ala Ile Glu Val Phe Glu Gln Tyr Val Arg Ser Cys Asn

                85   90   95

Met Asn Leu Lys His Phe His Leu Thr Ile Asp Ser Asn Leu Ala Asp
            100   105   110

Asn Ser Gly Gln Lys Tyr Gly Leu Gly Ser Ser Ala Ala Val Leu Val
        115   120   125

Ser Val Val Lys Ala Leu Asn Glu Phe Tyr Gly Leu Glu Leu Ser Asn
    130   135   140

Leu Tyr Ile Tyr Lys Leu Ala Val Ile Ala Asn Met Lys Leu Gln Ser
145   150   155   160

Leu Ser Ser Cys Gly Asp Ile Ala Val Ser Val Tyr Ser Gly Trp Leu
                165   170   175

Ala Tyr Ser Thr Phe Asp His Asp Trp Val Lys Gln Gln Met Glu Glu
            180   185   190

Thr Ser Val Asn Asp Val Leu Glu Lys Asn Trp Pro Gly Leu His Ile
        195   200   205

Glu Pro Leu Gln Ala Pro Glu Asn Met Glu Val Leu Ile Gly Trp Thr
    210   215   220

Gly Ser Pro Ala Ser Ser Pro His Leu Val Ser Glu Val Lys Arg Leu
225   230   235   240

Lys Ser Asp Pro Ser Phe Tyr Gly Asp Phe Leu Asp Gln Ser His Ala
                245   250   255

Cys Val Glu Ser Leu Ile Gln Ala Phe Lys Thr Asn Asn Ile Lys Gly
            260   265   270

Val Gln Lys Met Ile Arg Ile Asn Arg Arg Ile Ile Gln Ser Met Asp
        275   280   285

Asn Glu Ala Ser Val Glu Ile Glu Thr Asp Lys Leu Lys Lys Leu Cys
    290   295   300

Asp Val Gly Glu Lys His Gly Gly Ala Ser Lys Thr Ser Gly Ala Gly
305   310   315   320

Gly Gly Asp Cys Gly Ile Thr Ile Ile Asn Lys Val Ile Asp Lys Asn
                325   330   335

Ile Ile Tyr Asn Glu Trp Gln Met Asn Asp Ile Lys Pro Leu Lys Phe
            340   345   350

Lys Ile Tyr His Gly Gln
        355

<210> 111

<211> 358

<212> PRT

<213> Staphylococcus aureus

<400> 111

Met Ile Gln Val Lys Ala Pro Gly Lys Leu Tyr Ile Ala Gly Glu Tyr
1 5 10 15

Ala Val Thr Glu Pro Gly Tyr Lys Ser Val Leu Ile Ala Leu Asp Arg
20 25 30

Phe Val Thr Ala Thr Ile Glu Glu Ala Thr Gln Tyr Lys Gly Thr Ile
35 40 45

His Ser Lys Ala Leu His His Asn Pro Val Thr Phe Ser Arg Asp Glu
50 55 60

Asp Ser Ile Val Ile Ser Asp Pro His Ala Ala Lys Gln Leu Asn Tyr
65 70 75 80

Val Val Thr Ala Ile Glu Ile Phe Glu Gln Tyr Ala Lys Ser Cys Asp
85 90 95

Ile Ala Met Lys His Phe His Leu Thr Ile Asp Ser Asn Leu Asp Asp
100 105 110

Ser Asn Gly His Lys Tyr Gly Leu Gly Ser Ser Ala Ala Val Leu Val
115 120 125

Ser Val Ile Lys Val Leu Asn Glu Phe Tyr Asp Met Lys Leu Ser Asn
130 135 140

Leu Tyr Ile Tyr Lys Leu Ala Val Ile Ala Asn Met Lys Leu Gln Ser
145 150 155 160

Leu Ser Ser Cys Gly Asp Ile Ala Val Ser Val Tyr Ser Gly Trp Leu
165 170 175

Ala Tyr Ser Thr Phe Asp His Glu Trp Val Lys His Gln Ile Glu Asp
180 185 190

Thr Thr Val Glu Glu Val Leu Ile Lys Asn Trp Pro Gly Leu His Ile
195 200 205

Glu Pro Leu Gln Ala Pro Glu Asn Met Glu Val Leu Ile Gly Trp Thr
210 215 220

Gly Ser Pro Ala Ser Ser Pro His Phe Val Ser Glu Val Lys Arg Leu
225 230 235 240

Lys Ser Asp Pro Ser Phe Tyr Gly Asp Phe Leu Glu Asp Ser His Arg
245 250 255

Cys Val Glu Lys Leu Ile His Ala Phe Lys Thr Asn Asn Ile Lys Gly

260 265 270

Val Gln Lys Met Val Arg Gln Asn Arg Thr Ile Ile Gln Arg Met Asp
275 280 285

Lys Glu Ala Thr Val Asp Ile Glu Thr Glu Lys Leu Lys Tyr Leu Cys
290 295 300

Asp Ile Ala Glu Lys Tyr His Gly Ala Ser Lys Thr Ser Gly Ala Gly
305 310 315 320

Gly Gly Asp Cys Gly Ile Thr Ile Ile Asn Lys Asp Val Asp Lys Glu
325 330 335

Lys Ile Tyr Asp Glu Trp Thr Lys His Gly Ile Lys Pro Leu Lys Phe
340 345 350

Asn Ile Tyr His Gly Gln
355

<210> 112

<211> 374

<212> PRT

<213> Streptomyces sp. CL190

<400> 112

Met Thr Thr Gly Gln Arg Thr Ile Val Arg His Ala Pro Gly Lys Leu
1 5 10 15

Phe Val Ala Gly Glu Tyr Ala Val Val Asp Pro Gly Asn Pro Ala Ile
20 25 30

Leu Val Ala Val Asp Arg His Ile Ser Val Thr Val Ser Asp Ala Asp
35 40 45

Ala Asp Thr Gly Ala Ala Asp Val Val Ile Ser Ser Asp Leu Gly Pro
50 55 60

Gln Ala Val Gly Trp Arg Trp His Asp Gly Arg Leu Val Val Arg Asp
65 70 75 80

Pro Asp Asp Gly Gln Gln Ala Arg Ser Ala Leu Ala His Val Val Ser
85 90 95

Ala Ile Glu Thr Val Gly Arg Leu Leu Gly Glu Arg Gly Gln Lys Val
100 105 110

Pro Ala Leu Thr Leu Ser Val Ser Ser Arg Leu His Glu Asp Gly Arg
115 120 125

Lys Phe Gly Leu Gly Ser Ser Gly Ala Val Thr Val Ala Thr Val Ala

130 135 140

Ala Val Ala Ala Phe Cys Gly Leu Glu Leu Ser Thr Asp Glu Arg Phe
145 150 155 160

Arg Leu Ala Met Leu Ala Thr Ala Glu Leu Asp Pro Lys Gly Ser Gly
165 170 175

Gly Asp Leu Ala Ala Ser Thr Trp Gly Gly Trp Ile Ala Tyr Gln Ala
180 185 190

Pro Asp Arg Ala Phe Val Leu Asp Leu Ala Arg Arg Val Gly Val Asp
195 200 205

Arg Thr Leu Lys Ala Pro Trp Pro Gly His Ser Val Arg Arg Leu Pro
210 215 220

Ala Pro Lys Gly Leu Thr Leu Glu Val Gly Trp Thr Gly Glu Pro Ala
225 230 235 240

Ser Thr Ala Ser Leu Val Ser Asp Leu His Arg Arg Thr Trp Arg Gly
245 250 255

Ser Ala Ser His Gln Arg Phe Val Glu Thr Thr Thr Asp Cys Val Arg
260 265 270

Ser Ala Val Thr Ala Leu Glu Ser Gly Asp Asp Thr Ser Leu Leu His
275 280 285

Glu Ile Arg Arg Ala Arg Gln Glu Leu Ala Arg Leu Asp Asp Glu Val
290 295 300

Gly Leu Gly Ile Phe Thr Pro Lys Leu Thr Ala Leu Cys Asp Ala Ala
305 310 315 320

Glu Ala Val Gly Gly Ala Ala Lys Pro Ser Gly Ala Gly Gly Gly Asp
325 330 335

Cys Gly Ile Ala Leu Leu Asp Ala Glu Ala Ser Arg Asp Ile Thr His
340 345 350

Val Arg Gln Arg Trp Glu Thr Ala Gly Val Leu Pro Leu Pro Leu Thr
355 360 365

Pro Ala Leu Glu Gly Ile
370

<210> 113

<211> 360

<212> PRT

<213> Streptomyces griseolosporeus

<400> 113

Met Thr Gly Pro Arg Ala Val Thr Arg Arg Ala Pro Gly Lys Leu Phe
1 5 10 15

Val Ala Gly Glu Tyr Ala Val Val Glu Pro Gly Asn Arg Ala Ile Leu
20 25 30

Val Ala Val Asp Arg Tyr Val Thr Val Thr Val Ser Asp Gly Ala Ala
35 40 45

Pro Gly Val Val Val Ser Ser Asp Ile Gly Ala Gly Pro Val His His
50 55 60

Pro Trp Gln Asp Gly Arg Leu Thr Gly Gly Thr Gly Thr Pro His Val
65 70 75 80

Val Ala Ala Val Glu Thr Val Ala Arg Leu Leu Ala Glu Arg Gly Arg
85 90 95

Ser Val Pro Pro Leu Gly Trp Ser Ile Ser Ser Thr Leu His Glu Asp
100 105 110

Gly Arg Lys Phe Gly Leu Gly Ser Ser Gly Ala Val Thr Val Ala Thr
115 120 125

Val Ser Ala Val Ala Ala His Cys Gly Leu Glu Leu Thr Ala Glu Glu
130 135 140

Arg Phe Arg Thr Ala Leu Ile Ala Ser Ala Arg Ile Asp Pro Arg Gly
145 150 155 160

Ser Gly Gly Asp Ile Ala Thr Ser Thr Trp Gly Gly Trp Ile Ala Tyr
165 170 175

Arg Ala Pro Asp Arg Asp Ala Val Leu Asp Leu Thr Arg Arg Gln Gly
180 185 190

Val Asp Glu Ala Leu Arg Ala Pro Trp Pro Gly Phe Ser Val Arg Leu
195 200 205

Ser Pro Pro Arg Asn Leu Cys Leu Glu Val Gly Trp Thr Gly Asn Pro
210 215 220

Val Ser Thr Thr Ser Leu Leu Thr Asp Leu His Arg Arg Thr Trp Arg
225 230 235 240

Gly Ser Pro Ala Tyr Arg Arg Tyr Val Gly Ala Thr Gly Glu Leu Val
245 250 255

Asp Ala Ala Val Ile Ala Leu Glu Asp Gly Asp Thr Glu Gly Leu Leu
260 265 270

Arg Gln Val Arg Arg Ala Arg His Glu Met Val Arg Leu Asp Asp Glu
275 280 285

Val Gly Leu Gly Ile Phe Thr Pro Glu Leu Thr Ala Leu Cys Ala Ile

290 295 300

Ala Glu Arg Ala Gly Ala Ala Lys Pro Ser Gly Ala Gly Gly Gly Asp
305 310 315 320

Cys Gly Ile Ala Leu Leu Asp Ala Glu Ala Arg Tyr Asp Arg Ser Pro
325 330 335

Leu His Arg Gln Trp Ala Ala Ala Gly Val Leu Pro Leu Leu Val Ser
340 345 350

Pro Ala Thr Glu Gly Val Glu Glu
355 360

<210> 114

<211> 317

<212> PRT

<213> Borrelia burgdorferi

<400> 114

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Glu Tyr Thr Ile Leu Glu Glu Lys Gly Leu Gly Leu Ala Ile Ala Ile
20 25 30

Asn Lys Arg Ala Phe Phe Ser Phe Lys Lys Ser Asp Ser Trp Arg Phe
35 40 45

Phe Ser Lys Lys Lys Lys Ile Asp Asp Phe Ser Leu Ile Glu Asn Arg
50 55 60

Ser Asp Phe Val Phe Lys Met Phe Ala Tyr Leu Ser Gln Asn Cys Phe
65 70 75 80

Phe Asn Leu Glu Asn Phe Ala Tyr Asp Val Tyr Ile Asp Thr Ser Asn
85 90 95

Phe Phe Phe Asn Asp Gly Thr Lys Lys Gly Phe Gly Ser Ser Ala Val
100 105 110

Val Ala Ile Gly Ile Val Cys Gly Leu Phe Leu Ile His Asn Ala Thr
115 120 125

Asn Val Val Glu Lys Gly Glu Ile Phe Lys Tyr Cys Leu Glu Ala Tyr
130 135 140

Arg Tyr Ser Gln Gly Gly Ile Gly Ser Gly Tyr Asp Ile Ala Thr Ser
145 150 155 160

Ile Phe Gly Gly Val Ile Glu Phe Glu Gly Gly Phe Asn Pro Lys Cys

165 170 175

Arg Gln Leu Gly Ala Val Glu Phe Asn Asp Phe Tyr Leu Met Gln Gly
180 185 190

Leu Gln Ala Ile Lys Thr Thr Thr Ser Ile Cys Glu Tyr Asn Lys His
195 200 205

Arg Asn Ser Ile Leu Asp Phe Ile Leu Lys Cys Asn Leu Glu Met Lys
210 215 220

Lys Leu Val Leu Asn Ala Ser Asn Ser Lys Ser Ala Leu Ile Ser Ser
225 230 235 240

Leu Arg Arg Ala Lys Glu Leu Gly Leu Ala Ile Gly Glu Ala Ile Gly
245 250 255

Val Ser Ala Ala Leu Pro Ser Ser Phe Asp His Leu Leu Gly Gln Cys
260 265 270

Asp Leu Ile Lys Ala Leu Gly Ala Gly Asn Glu Thr Phe Leu Val Tyr
275 280 285

Arg Pro Asn Ile Glu Ala Phe Asn Leu Ser Lys Ile Ile Ser Ile Val
290 295 300

Leu Glu Asn Glu Gly Ile Lys Phe Glu Ser Asp Lys Cys
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<210> 116

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<210> 147

<211> 26

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<213> synthetic construct

<400> 147
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<210> 148

<211> 32

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<400> 148
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<210> 157

<211> 1612

<212> DNA

<213> Paracoccus sp. R114

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<222> (59)..(292)

<223> XseB

<220>

<221> CDS

<222> (1185)..(1610)

<223> IspA

<220>

<221> CDS

<222> (295)..(1158)

<223> Dxs

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Met Ser Asp Ile Gln Thr Leu Ser Phe Glu Glu Ala Met Arg Glu Leu
1 5 10 15

gag gcg acc gtc ggc aag ctg gaa acc ggc gag gcg acg ctc gag gac 154
Glu Ala Thr Val Gly Lys Leu Glu Thr Gly Glu Ala Thr Leu Glu Asp
20 25 30

tcc atc gcg ctc tat gaa cgc ggg gcg gcg ctg cgc gcc cat tgc gaa 202
Ser Ile Ala Leu Tyr Glu Arg Gly Ala Ala Leu Arg Ala His Cys Glu
35 40 45

acc cgc ctg cgc gag gcc gag gag cgg gtc gag aag atc acc ctg gcc 250
Thr Arg Leu Arg Glu Ala Glu Glu Arg Val Glu Lys Ile Thr Leu Ala
50 55 60

gcg aac ggg cag ccg tcc gga acc gag ccc gcc gag ggc ctg tg atg 297
Ala Asn Gly Gln Pro Ser Gly Thr Glu Pro Ala Glu Gly Leu Met
65 70 75

cag gcc cgc ctg gcc gag atc cgg ccc ctg gtc gag gcc gag ctg aac 345
Gln Ala Arg Leu Ala Glu Ile Arg Pro Leu Val Glu Ala Glu Leu Asn
80 85 90 95

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gcc gcc atc gac gcg ctg ccc gcg ggc gat ctg tcg gat gcg atg cgc      393
Ala Ala Ile Asp Ala Leu Pro Ala Gly Asp Leu Ser Asp Ala Met Arg
                100                 105                 110

tat gcc gtg cag ggc ggc aag cgg ctg cgc gcg ttc ctg gtg atg gag      441
Tyr Ala Val Gln Gly Gly Lys Arg Leu Arg Ala Phe Leu Val Met Glu
            115                 120                 125

tcg gcg cgc ctg cac ggg ctg gac gac gac gca tcg ctg ccc gtc gcc      489
Ser Ala Arg Leu His Gly Leu Asp Asp Asp Ala Ser Leu Pro Val Ala
        130                 135                 140

gcc gcg gtc gag gcg ctg cac gcc tac agc ttg gtc cat gac gac ctg      537
Ala Ala Val Glu Ala Leu His Ala Tyr Ser Leu Val His Asp Asp Leu
    145                 150                 155

ccc gcg atg gat gac gac gac ctg cgg cgc ggt cag ccc acc gtc cac      585
Pro Ala Met Asp Asp Asp Asp Leu Arg Arg Gly Gln Pro Thr Val His
160                 165                 170                 175

gtc aaa tgg acc gag gcg acc gcg atc ctt gcg ggc gat gcg ctg cag      633
Val Lys Trp Thr Glu Ala Thr Ala Ile Leu Ala Gly Asp Ala Leu Gln
                180                 185                 190

acg ctg gcc ttc cag ctg ctg gcc gat ccg cgc gtg ggc gac gat gcg      681
Thr Leu Ala Phe Gln Leu Leu Ala Asp Pro Arg Val Gly Asp Asp Ala
            195                 200                 205

gcg cgg atg cgg ctg gtc ggt tcg ctg gcg cag gca tcg ggg gct gcg      729
Ala Arg Met Arg Leu Val Gly Ser Leu Ala Gln Ala Ser Gly Ala Ala
        210                 215                 220

ggc atg gtc tgg ggc cag gcg ctg gac atc gcg gcc gag acc tcg ggc      777
Gly Met Val Trp Gly Gln Ala Leu Asp Ile Ala Ala Glu Thr Ser Gly
    225                 230                 235

gtg ccg ctg gat ctg gac gcg atc atc cgc ctg cag ggt ggc aag acc      825
Val Pro Leu Asp Leu Asp Ala Ile Ile Arg Leu Gln Gly Gly Lys Thr
240                 245                 250                 255

ggc gcg ctg atc cgc ttt gcc gcg acc gcc ggg ccg ctg atg gcg ggg      873
Gly Ala Leu Ile Arg Phe Ala Ala Thr Ala Gly Pro Leu Met Ala Gly
                260                 265                 270

gcg gac cct gcc gcg ctg gac gat tat gcg cag gcc gtc ggg ctg gcc      921
Ala Asp Pro Ala Ala Leu Asp Asp Tyr Ala Gln Ala Val Gly Leu Ala
            275                 280                 285

ttc cag atc gcg gac gac atc ctg gac gtc gag ggc tgc gag gcc gcg      969
Phe Gln Ile Ala Asp Asp Ile Leu Asp Val Glu Gly Cys Glu Ala Ala
        290                 295                 300

acc ggc aag cgc gtc ggc aag gat gcg gat gcc aac aag gcg acc ttc     1017
Thr Gly Lys Arg Val Gly Lys Asp Ala Asp Ala Asn Lys Ala Thr Phe
    305                 310                 315

gtc tcg ctg ctg ggc ctc gag ggg gcg cgg tcc gag gcg cgt cgc ctg     1065
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Val Ser Leu Leu Gly Leu Glu Gly Ala Arg Ser Glu Ala Arg Arg Leu
320 325 330 335

gcc gat gcg ggg cag gac gcg ctg gcg ggt tac ggc gat gct gcg ggg 1113
Ala Asp Ala Gly Gln Asp Ala Leu Ala Gly Tyr Gly Asp Ala Ala Gly
340 345 350

aac ctt cgg gac ctg gcg cgc ttc gtg atc gaa cgc gac agc tga 1158
Asn Leu Arg Asp Leu Ala Arg Phe Val Ile Glu Arg Asp Ser
355 360 365

tcgccgcctt cccgccaagg ggcaag atg atg acc gac gga ccc gca acc ccg 1211
Met Met Thr Asp Gly Pro Ala Thr Pro
370

atc ctg gac cgc gtc cag cag cca tcc gac ctg gca tcg ctg gac gat 1259
Ile Leu Asp Arg Val Gln Gln Pro Ser Asp Leu Ala Ser Leu Asp Asp
375 380 385 390

gcg cag ctg cgc ctg ctg gcg gac gag ctg cgg gcc gag acc atc gac 1307
Ala Gln Leu Arg Leu Leu Ala Asp Glu Leu Arg Ala Glu Thr Ile Asp
395 400 405

atc gtc agc cgc acg ggc ggt cac ctg ggc gcg ggg ctg ggc gtg gtc 1355
Ile Val Ser Arg Thr Gly Gly His Leu Gly Ala Gly Leu Gly Val Val
410 415 420

gaa ctg acg gtc gcc ctg cac gcc gtc ttt cgg gcg ccg cgc gac aag 1403
Glu Leu Thr Val Ala Leu His Ala Val Phe Arg Ala Pro Arg Asp Lys
425 430 435

atc gtc tgg gac gtg ggg cat caa tgc tat ccc cac aag atc ctg acg 1451
Ile Val Trp Asp Val Gly His Gln Cys Tyr Pro His Lys Ile Leu Thr
440 445 450

ggc agg cgg gac cgg atg cgc acg ctg cgc atg ggc ggc ggg ctg tcg 1499
Gly Arg Arg Asp Arg Met Arg Thr Leu Arg Met Gly Gly Gly Leu Ser
455 460 465 470

ggg ttc acc aag cgg cag gaa agc gcg ttc gat ccg ttc ggt gcg ggg 1547
Gly Phe Thr Lys Arg Gln Glu Ser Ala Phe Asp Pro Phe Gly Ala Gly
475 480 485

cac agc tcg acc tcg atc tcg gcg gcg ctg ggc ttc gcg atg gcg cgt 1595
His Ser Ser Thr Ser Ile Ser Ala Ala Leu Gly Phe Ala Met Ala Arg
490 495 500

gaa ctt ggc ggg gat cc 1612
Glu Leu Gly Gly Asp
505

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<211> 78

<212> PRT

<213> Paracoccus sp. R114

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Glu Ala Thr Val Gly Lys Leu Glu Thr Gly Glu Ala Thr Leu Glu Asp
20 25 30

Ser Ile Ala Leu Tyr Glu Arg Gly Ala Ala Leu Arg Ala His Cys Glu
35 40 45

Thr Arg Leu Arg Glu Ala Glu Glu Arg Val Glu Lys Ile Thr Leu Ala
50 55 60

Ala Asn Gly Gln Pro Ser Gly Thr Glu Pro Ala Glu Gly Leu
65 70 75

<210> 159

<211> 287

<212> PRT

<213> Paracoccus sp. R114

<400> 159

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Asn Ala Ala Ile Asp Ala Leu Pro Ala Gly Asp Leu Ser Asp Ala Met
20 25 30

Arg Tyr Ala Val Gln Gly Gly Lys Arg Leu Arg Ala Phe Leu Val Met
35 40 45

Glu Ser Ala Arg Leu His Gly Leu Asp Asp Asp Ala Ser Leu Pro Val
50 55 60

Ala Ala Ala Val Glu Ala Leu His Ala Tyr Ser Leu Val His Asp Asp
65 70 75 80

Leu Pro Ala Met Asp Asp Asp Asp Leu Arg Arg Gly Gln Pro Thr Val
85 90 95

His Val Lys Trp Thr Glu Ala Thr Ala Ile Leu Ala Gly Asp Ala Leu
100 105 110

Gln Thr Leu Ala Phe Gln Leu Leu Ala Asp Pro Arg Val Gly Asp Asp
115 120 125

Ala Ala Arg Met Arg Leu Val Gly Ser Leu Ala Gln Ala Ser Gly Ala
130 135 140

Ala Gly Met Val Trp Gly Gln Ala Leu Asp Ile Ala Ala Glu Thr Ser
145 150 155 160

Gly Val Pro Leu Asp Leu Asp Ala Ile Ile Arg Leu Gln Gly Gly Lys
165 170 175

Thr Gly Ala Leu Ile Arg Phe Ala Ala Thr Ala Gly Pro Leu Met Ala
180 185 190

Gly Ala Asp Pro Ala Ala Leu Asp Asp Tyr Ala Gln Ala Val Gly Leu
195 200 205

Ala Phe Gln Ile Ala Asp Asp Ile Leu Asp Val Glu Gly Cys Glu Ala
210 215 220

Ala Thr Gly Lys Arg Val Gly Lys Asp Ala Asp Ala Asn Lys Ala Thr
225 230 235 240

Phe Val Ser Leu Leu Gly Leu Glu Gly Ala Arg Ser Glu Ala Arg Arg
245 250 255

Leu Ala Asp Ala Gly Gln Asp Ala Leu Ala Gly Tyr Gly Asp Ala Ala
260 265 270

Gly Asn Leu Arg Asp Leu Ala Arg Phe Val Ile Glu Arg Asp Ser
275 280 285

<210> 160

<211> 142

<212> PRT

<213> Paracoccus sp. R114

<400> 160

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1 5 10 15

Pro Ser Asp Leu Ala Ser Leu Asp Asp Ala Gln Leu Arg Leu Leu Ala
20 25 30

Asp Glu Leu Arg Ala Glu Thr Ile Asp Ile Val Ser Arg Thr Gly Gly
35 40 45

His Leu Gly Ala Gly Leu Gly Val Val Glu Leu Thr Val Ala Leu His
50 55 60

Ala Val Phe Arg Ala Pro Arg Asp Lys Ile Val Trp Asp Val Gly His
65 70 75 80

Gln Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Arg Met Arg
85 90 95

Thr Leu Arg Met Gly Gly Gly Leu Ser Gly Phe Thr Lys Arg Gln Glu
100 105 110

Ser Ala Phe Asp Pro Phe Gly Ala Gly His Ser Ser Thr Ser Ile Ser
115 120 125

Ala Ala Leu Gly Phe Ala Met Ala Arg Glu Leu Gly Gly Asp
130 135 140

<210> 161

<211> 6

<212> PRT

<213> Bradyrhizobium japonicum

<400> 161

Val His Asp Asp Leu Pro
1 5

<210> 162

<211> 6

<212> PRT

<213> Rhizobium sp. strain NGR234

<400> 162

Val His Asp Asp Leu Pro
1 5

<210> 163

<211> 6

<212> PRT

<213> Bacillus stearothermophilus

<400> 163

Ile His Asp Asp Leu Pro
1 5

<210> 164

<211> 6

<212> PRT

<213> Bacillus subtilis

<400> 164

Ile His Asp Asp Leu Pro
1 5

<210> 165

<211> 6

<212> PRT

<213> Escherichia coli

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<210> 166

<211> 6

<212> PRT

<213> Haemophilus influenzae

<400> 166

Ile His Asp Asp Leu Pro
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<210> 167

<211> 16

<212> DNA

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<400> 167
tccaygayga yctgcc 16

<210> 168

<211> 5

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<213> Bradyrhizobium japonicum

<400> 168

Asp Asp Ile Leu Asp
1 5

<210> 169

<211> 5

<212> PRT

<213> Rhizobium sp. strain NGR234

<400> 169

Asp Asp Ile Leu Asp
1 5

<210> 170

<211> 5

<212> PRT

<213> Bacillus stearothermophilus

<400> 170

Asp Asp Ile Leu Asp
1 5

<210> 171

<211> 5

<212> PRT

<213> Bacillus subtilis

<400> 171

Asp Asp Ile Leu Asp
1 5

<210> 172

<211> 5

<212> PRT

<213> Escherichia coli

<400> 172

Asp Asp Ile Leu Asp
1 5

<210> 173

<211> 5

<212> PRT

<213> Haemophilus influenzae

<400> 173

Asp Asp Ile Leu Asp
1 5

<210> 174

<211> 15

<212> DNA

<213> synthetic construct

<400> 174
gaygayatcc tggay 15

<210> 175

<211> 1176

<212> DNA

<213> Paracoccus sp. R1534

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<221> CDS

<222> (1)..(1173)

<223> acety-CoA acetyltransferase

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1 5 10 15

ttc cag ggc gat ctt gcc gcg atg gat gcc ccg acc ctt ggc gcg gcc 96
Phe Gln Gly Asp Leu Ala Ala Met Asp Ala Pro Thr Leu Gly Ala Ala
20 25 30

gcg atc cgc gcc gcg ctg aac ggc ctg tcg ccc gac atg gtg gac gag 144
Ala Ile Arg Ala Ala Leu Asn Gly Leu Ser Pro Asp Met Val Asp Glu
35 40 45

gtg ctg atg ggc tgc gtc ctg ccc gcg ggc cag ggt cag gca ccg gca 192
Val Leu Met Gly Cys Val Leu Pro Ala Gly Gln Gly Gln Ala Pro Ala
50 55 60

cgt cag gcg gcg ctt gac gcc gga ctg ccg ctg tcg gcg ggc gcg acc 240
Arg Gln Ala Ala Leu Asp Ala Gly Leu Pro Leu Ser Ala Gly Ala Thr
65 70 75 80

acc atc aac aag atg tgc gga tcg ggc atg aag gcc gcg atg ctg ggc 288
Thr Ile Asn Lys Met Cys Gly Ser Gly Met Lys Ala Ala Met Leu Gly
85 90 95

cat gac ctg atc gcc gcg gga tcg gcg ggc atc gtc gtc gcc ggc ggg 336
His Asp Leu Ile Ala Ala Gly Ser Ala Gly Ile Val Val Ala Gly Gly
100 105 110

atg gag agc atg tcg aac gcc ccc tac ctg ctg ccc aag gcg cgg tcg 384
Met Glu Ser Met Ser Asn Ala Pro Tyr Leu Leu Pro Lys Ala Arg Ser
115 120 125

ggg atg cgc atg ggc cat gac cgt gtg ctg gat cac atg ttc ctc gac 432
Gly Met Arg Met Gly His Asp Arg Val Leu Asp His Met Phe Leu Asp
130 135 140

ggg ttg gag gac gcc tat gac aag ggc cgc ctg atg ggc acc ttc gcc 480
Gly Leu Glu Asp Ala Tyr Asp Lys Gly Arg Leu Met Gly Thr Phe Ala
145 150 155 160

gag gat tgc gcc ggc gat cac ggt ttc acc cgc gag gcg cag gac gac 528
Glu Asp Cys Ala Gly Asp His Gly Phe Thr Arg Glu Ala Gln Asp Asp
165 170 175

tat gcg ctg acc agc ctg gcc cgc gcg cag gac gcc atc gcc agc ggt 576
Tyr Ala Leu Thr Ser Leu Ala Arg Ala Gln Asp Ala Ile Ala Ser Gly
180 185 190

gcc ttc gcc gcc gag atc gcg ccc gtg acc gtc acg gca cgc aag gtg 624
Ala Phe Ala Ala Glu Ile Ala Pro Val Thr Val Thr Ala Arg Lys Val
195 200 205

cag acc acc gtc gat acc gac gag atg ccc ggc aag gcc cgc ccc gag 672
Gln Thr Thr Val Asp Thr Asp Glu Met Pro Gly Lys Ala Arg Pro Glu
210 215 220

aag atc ccc cat ctg aag ccc gcc ttc cgt gac ggt ggc acg gtc acg 720
Lys Ile Pro His Leu Lys Pro Ala Phe Arg Asp Gly Gly Thr Val Thr
225 230 235 240

gcg gcg aac agc tcg tcg atc tcg gac ggg gcg gcg gcg ctg gtg atg 768
Ala Ala Asn Ser Ser Ser Ile Ser Asp Gly Ala Ala Ala Leu Val Met
245 250 255

atg cgc cag tcg cag gcc gag aag ctg ggc ctg acg ccg atc gcg cgg 816
Met Arg Gln Ser Gln Ala Glu Lys Leu Gly Leu Thr Pro Ile Ala Arg
260 265 270

atc atc ggt cat gcg acc cat gcc gac cgt ccc ggc ctg ttc ccg acg 864
Ile Ile Gly His Ala Thr His Ala Asp Arg Pro Gly Leu Phe Pro Thr
275 280 285

gcc ccc atc ggc gcg atg cgc aag ctg ctg gac cgc acg gac acc cgc 912
Ala Pro Ile Gly Ala Met Arg Lys Leu Leu Asp Arg Thr Asp Thr Arg
290 295 300

ctt ggc gat tac gac ctg ttc gag gtg aac gag gca ttc gcc gtc gtc 960
Leu Gly Asp Tyr Asp Leu Phe Glu Val Asn Glu Ala Phe Ala Val Val
305 310 315 320

gcc atg atc gcg atg aag gag ctt ggc ctg cca cac gat gcc acg aac 1008
Ala Met Ile Ala Met Lys Glu Leu Gly Leu Pro His Asp Ala Thr Asn
325 330 335

atc aac ggc ggg gcc tgc gcg ctt ggg cat ccc atc ggc gcg tcg ggg 1056
Ile Asn Gly Gly Ala Cys Ala Leu Gly His Pro Ile Gly Ala Ser Gly
340 345 350

gcg cgg atc atg gtc acg ctg ctg aac gcg atg gcg gcg cgg ggc gcg 1104
Ala Arg Ile Met Val Thr Leu Leu Asn Ala Met Ala Ala Arg Gly Ala
355 360 365

acg cgc ggg gcc gca tcc gtc tgc atc ggc ggg ggc gag gcg acg gcc 1152
Thr Arg Gly Ala Ala Ser Val Cys Ile Gly Gly Gly Glu Ala Thr Ala
370 375 380

atc gcg ctg gaa cgg ctg agc taa 1176
Ile Ala Leu Glu Arg Leu Ser
385 390

<210> 176

<211> 391

<212> PRT

<213> Paracoccus sp. R1534

<400> 176

Met Asp Pro Ile Val Ile Thr Gly Ala Met Arg Thr Pro Met Gly Ala
1 5 10 15

Phe Gln Gly Asp Leu Ala Ala Met Asp Ala Pro Thr Leu Gly Ala Ala
20 25 30

Ala Ile Arg Ala Ala Leu Asn Gly Leu Ser Pro Asp Met Val Asp Glu
35 40 45

Val Leu Met Gly Cys Val Leu Pro Ala Gly Gln Gly Gln Ala Pro Ala
50 55 60

Arg Gln Ala Ala Leu Asp Ala Gly Leu Pro Leu Ser Ala Gly Ala Thr
65 70 75 80

Thr Ile Asn Lys Met Cys Gly Ser Gly Met Lys Ala Ala Met Leu Gly
85 90 95

His Asp Leu Ile Ala Ala Gly Ser Ala Gly Ile Val Val Ala Gly Gly
100 105 110

Met Glu Ser Met Ser Asn Ala Pro Tyr Leu Leu Pro Lys Ala Arg Ser
115 120 125

Gly Met Arg Met Gly His Asp Arg Val Leu Asp His Met Phe Leu Asp
130 135 140

Gly Leu Glu Asp Ala Tyr Asp Lys Gly Arg Leu Met Gly Thr Phe Ala
145 150 155 160

Glu Asp Cys Ala Gly Asp His Gly Phe Thr Arg Glu Ala Gln Asp Asp
165 170 175

Tyr Ala Leu Thr Ser Leu Ala Arg Ala Gln Asp Ala Ile Ala Ser Gly
180 185 190

Ala Phe Ala Ala Glu Ile Ala Pro Val Thr Val Thr Ala Arg Lys Val
195 200 205

Gln Thr Thr Val Asp Thr Asp Glu Met Pro Gly Lys Ala Arg Pro Glu
210 215 220

Lys Ile Pro His Leu Lys Pro Ala Phe Arg Asp Gly Gly Thr Val Thr
225 230 235 240

Ala Ala Asn Ser Ser Ser Ile Ser Asp Gly Ala Ala Ala Leu Val Met
245 250 255

Met Arg Gln Ser Gln Ala Glu Lys Leu Gly Leu Thr Pro Ile Ala Arg
260 265 270

Ile Ile Gly His Ala Thr His Ala Asp Arg Pro Gly Leu Phe Pro Thr

275 280 285

Ala Pro Ile Gly Ala Met Arg Lys Leu Leu Asp Arg Thr Asp Thr Arg
290 295 300

Leu Gly Asp Tyr Asp Leu Phe Glu Val Asn Glu Ala Phe Ala Val Val
305 310 315 320

Ala Met Ile Ala Met Lys Glu Leu Gly Leu Pro His Asp Ala Thr Asn
325 330 335

Ile Asn Gly Gly Ala Cys Ala Leu Gly His Pro Ile Gly Ala Ser Gly
340 345 350

Ala Arg Ile Met Val Thr Leu Leu Asn Ala Met Ala Ala Arg Gly Ala
355 360 365

Thr Arg Gly Ala Ala Ser Val Cys Ile Gly Gly Gly Glu Ala Thr Ala
370 375 380

Ile Ala Leu Glu Arg Leu Ser
385 390

<210> 177

<211> 1980

<212> DNA

<213> Paracoccus sp. R114

<220>

<221> CDS

<222> (1)..(1170)

<223> phaA

<220>

<221> misc_feature

<222> (1179)..(1194)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<220>

<221> misc_feature

<222> (1196)..(1210)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<220>

<221> CDS

<222> (1258)..(1980)

<223> phaB

<400> 177

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atg acc aaa gcc gta atc gta tct gcc gca cgt acc ccc gtc ggc agc      48
Met Thr Lys Ala Val Ile Val Ser Ala Ala Arg Thr Pro Val Gly Ser
1               5                   10                  15

ttc atg ggc gca ttc gcc aat gtc ccc gca cat gat ctg ggc gcc gcc      96
Phe Met Gly Ala Phe Ala Asn Val Pro Ala His Asp Leu Gly Ala Ala
            20                  25                  30

gtc ctg cgc gag gtc gtg gcc cgc gcc ggt gtc gac ccc gcc gag gtc     144
Val Leu Arg Glu Val Val Ala Arg Ala Gly Val Asp Pro Ala Glu Val
        35                  40                  45

agc gag acg atc ctg ggc cag gtg ctg acc gcc gcg cag ggc cag aac     192
Ser Glu Thr Ile Leu Gly Gln Val Leu Thr Ala Ala Gln Gly Gln Asn
    50                  55                  60

ccc gcg cgc cag gcg cat atc aat gcg ggc ctg ccc aag gaa tcg gcg     240
Pro Ala Arg Gln Ala His Ile Asn Ala Gly Leu Pro Lys Glu Ser Ala
65                  70                  75                  80

gcg tgg ctc atc aac cag gtc tgc ggc tcg ggg ctg cgc gcc gtc gcg     288
Ala Trp Leu Ile Asn Gln Val Cys Gly Ser Gly Leu Arg Ala Val Ala
                85                  90                  95

ctg gcg gcg cag cag gtc atg ctg ggc gat gcg cag atc gtt ctg gcg     336
Leu Ala Ala Gln Gln Val Met Leu Gly Asp Ala Gln Ile Val Leu Ala
            100                 105                 110

ggg ggc cag gag agc atg tcg ctg tcg acc cat gcc gcc tat ctg cgc     384
Gly Gly Gln Glu Ser Met Ser Leu Ser Thr His Ala Ala Tyr Leu Arg
```

```
        115                 120                 125

gcg ggc cag aag atg ggc gac atg aag atg atc gac acc atg atc cgc      432
Ala Gly Gln Lys Met Gly Asp Met Lys Met Ile Asp Thr Met Ile Arg
    130                 135                 140

gac ggg ctg tgg gat gcc ttc aac ggc tat cac atg ggt cag acc gcc      480
Asp Gly Leu Trp Asp Ala Phe Asn Gly Tyr His Met Gly Gln Thr Ala
145                 150                 155                 160

gag aac gtg gcc gac cag tgg tcg atc agc cgc gac cag cag gac gaa      528
Glu Asn Val Ala Asp Gln Trp Ser Ile Ser Arg Asp Gln Gln Asp Glu
                165                 170                 175

ttc gcc ctg gct tcg cag aac aag gcc gag gcc gcg cag aat gcg ggc      576
Phe Ala Leu Ala Ser Gln Asn Lys Ala Glu Ala Ala Gln Asn Ala Gly
            180                 185                 190

cgc ttc gat gac gaa atc gtc gcc tat acc gtc aag ggc cgc aag ggc      624
Arg Phe Asp Asp Glu Ile Val Ala Tyr Thr Val Lys Gly Arg Lys Gly
        195                 200                 205

gac acg gtc gtc gac aag gac gaa tac atc cgc cac ggc gcc acg atc      672
Asp Thr Val Val Asp Lys Asp Glu Tyr Ile Arg His Gly Ala Thr Ile
    210                 215                 220

gag ggc atg cag aag ctg cgc ccc gcc ttc acc aag gaa ggc tcg gtc      720
Glu Gly Met Gln Lys Leu Arg Pro Ala Phe Thr Lys Glu Gly Ser Val
225                 230                 235                 240

acg gcg ggc aac gcg tcg ggc ctg aac gac ggc gcg gcg gcc gtc atg      768
Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Gly Ala Ala Ala Val Met
                245                 250                 255

gtc atg tcc gag gac gag gcc gca cgc cgc ggg ctg acg ccg ctg gcg      816
Val Met Ser Glu Asp Glu Ala Ala Arg Arg Gly Leu Thr Pro Leu Ala
            260                 265                 270

cgc atc gcc tcc tat gcg acg gcg ggc ctc gac ccg gcg atc atg ggc      864
Arg Ile Ala Ser Tyr Ala Thr Ala Gly Leu Asp Pro Ala Ile Met Gly
        275                 280                 285

acc ggg ccg atc ccc tcc agc cgc aag gcg ctg gaa aag gcg ggc tgg      912
Thr Gly Pro Ile Pro Ser Ser Arg Lys Ala Leu Glu Lys Ala Gly Trp
    290                 295                 300

tcg gtc ggc gac ctg gac ctg gtc gag gcg aac gag gcc ttt gcc gcg      960
Ser Val Gly Asp Leu Asp Leu Val Glu Ala Asn Glu Ala Phe Ala Ala
305                 310                 315                 320

cag gcc tgc gcc gtg aac aag gac atg ggc tgg gat ccg tcc atc gtg     1008
Gln Ala Cys Ala Val Asn Lys Asp Met Gly Trp Asp Pro Ser Ile Val
                325                 330                 335

aac gtc aac ggc ggc gcg atc gcc atc ggc cac ccg atc ggc gcc tcg     1056
Asn Val Asn Gly Gly Ala Ile Ala Ile Gly His Pro Ile Gly Ala Ser
            340                 345                 350
```

```
ggg gcg cgg atc ctg aac acc ctg ctg ttc gag atg cag cgc cgc gac      1104
Gly Ala Arg Ile Leu Asn Thr Leu Leu Phe Glu Met Gln Arg Arg Asp
        355                 360                 365

gcc aag aag ggc ctt gcg acg ctg tgc atc ggc ggc ggc atg ggc gtc      1152
Ala Lys Lys Gly Leu Ala Thr Leu Cys Ile Gly Gly Gly Met Gly Val
    370                 375                 380

gcc atg tgc ctc gaa cgc tgaacgaccg gcgtgtgcgc aatttaattg             1200
Ala Met Cys Leu Glu Arg
385                 390

cgcacacgcc ccctgcaaag tagcaatgtt ttacgataac gaatgaaggg gggaatc      1257

atg tcc aag gta gca ctg gtc acc ggc gga tcg cgc ggc atc ggc gcc      1305
Met Ser Lys Val Ala Leu Val Thr Gly Gly Ser Arg Gly Ile Gly Ala
                395                 400                 405

gag atc tgc aag gcg ctt cag gcc gca ggc tat acc gtc gcc gcg aac      1353
Glu Ile Cys Lys Ala Leu Gln Ala Ala Gly Tyr Thr Val Ala Ala Asn
            410                 415                 420

tat gcc ggc aat gac gac gcg gcc aag gcc ttc acc gag gaa acc ggc      1401
Tyr Ala Gly Asn Asp Asp Ala Ala Lys Ala Phe Thr Glu Glu Thr Gly
        425                 430                 435

atc aag acc tac aag tgg tcg gtc gcc gat tac gat gcc tgc aag gcc      1449
Ile Lys Thr Tyr Lys Trp Ser Val Ala Asp Tyr Asp Ala Cys Lys Ala
    440                 445                 450

ggc atc gcc cag gtc gaa gag gat ctg ggc ccg atc gcc gtg ctg atc      1497
Gly Ile Ala Gln Val Glu Glu Asp Leu Gly Pro Ile Ala Val Leu Ile
455                 460                 465                 470

aac aat gcc ggg atc acc cgc gac gcg ccc ttc cac aag atg acg ccc      1545
Asn Asn Ala Gly Ile Thr Arg Asp Ala Pro Phe His Lys Met Thr Pro
                475                 480                 485

gag aag tgg aag gag gtc atc gac acc aac ctg acc ggc acc ttc aac      1593
Glu Lys Trp Lys Glu Val Ile Asp Thr Asn Leu Thr Gly Thr Phe Asn
            490                 495                 500

atg acc cat ccg gtc tgg ccg ggc atg cgc gaa cgc aag ttc gga cgc      1641
Met Thr His Pro Val Trp Pro Gly Met Arg Glu Arg Lys Phe Gly Arg
        505                 510                 515

gtc atc aac atc agc tcg atc aac ggg cag aag ggc cag ttc ggg cag      1689
Val Ile Asn Ile Ser Ser Ile Asn Gly Gln Lys Gly Gln Phe Gly Gln
    520                 525                 530

gcg aac tat gcc gcg gcc aag gcg ggc gac ctg ggc ttc acc aag tcg      1737
Ala Asn Tyr Ala Ala Ala Lys Ala Gly Asp Leu Gly Phe Thr Lys Ser
535                 540                 545                 550

ctg gcg cag gaa ggc gcg cgc aac aac atc acc gtc aac gcg atc tgc      1785
Leu Ala Gln Glu Gly Ala Arg Asn Asn Ile Thr Val Asn Ala Ile Cys
```

```
                555                 560                 565

ccc ggc tat atc gcg acg gac atg gtg atg gcc gtt ccc gaa cag gtc      1833
Pro Gly Tyr Ile Ala Thr Asp Met Val Met Ala Val Pro Glu Gln Val
            570                 575                 580

cgc gag ggg atc atc gcg cag atc ccc gtc ggc cgc ttg ggc gag ccg      1881
Arg Glu Gly Ile Ile Ala Gln Ile Pro Val Gly Arg Leu Gly Glu Pro
        585                 590                 595

tcc gag atc gcg cgc tgc gtg gtg ttc ctg gcc tcc gac gat gcg ggc      1929
Ser Glu Ile Ala Arg Cys Val Val Phe Leu Ala Ser Asp Asp Ala Gly
    600                 605                 610

ttc gtc aca ggc tcg acc atc acg gcg aat ggc ggc cag tac tac atc      1977
Phe Val Thr Gly Ser Thr Ile Thr Ala Asn Gly Gly Gln Tyr Tyr Ile
615                 620                 625                 630

tga                                                                  1980
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<210> 178

<211> 390

<212> PRT

<213> Paracoccus sp. R114

<220>

<221> misc_feature

<222> (1179)..(1194)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<220>

<221> misc_feature

<222> (1196)..(1210)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<400> 178

```
Met Thr Lys Ala Val Ile Val Ser Ala Ala Arg Thr Pro Val Gly Ser
1               5                   10                  15

Phe Met Gly Ala Phe Ala Asn Val Pro Ala His Asp Leu Gly Ala Ala
            20                  25                  30
```

Val Leu Arg Glu Val Val Ala Arg Ala Gly Val Asp Pro Ala Glu Val
35 40 45

Ser Glu Thr Ile Leu Gly Gln Val Leu Thr Ala Ala Gln Gly Gln Asn
50 55 60

Pro Ala Arg Gln Ala His Ile Asn Ala Gly Leu Pro Lys Glu Ser Ala
65 70 75 80

Ala Trp Leu Ile Asn Gln Val Cys Gly Ser Gly Leu Arg Ala Val Ala
85 90 95

Leu Ala Ala Gln Gln Val Met Leu Gly Asp Ala Gln Ile Val Leu Ala
100 105 110

Gly Gly Gln Glu Ser Met Ser Leu Ser Thr His Ala Ala Tyr Leu Arg
115 120 125

Ala Gly Gln Lys Met Gly Asp Met Lys Met Ile Asp Thr Met Ile Arg
130 135 140

Asp Gly Leu Trp Asp Ala Phe Asn Gly Tyr His Met Gly Gln Thr Ala
145 150 155 160

Glu Asn Val Ala Asp Gln Trp Ser Ile Ser Arg Asp Gln Gln Asp Glu
165 170 175

Phe Ala Leu Ala Ser Gln Asn Lys Ala Glu Ala Ala Gln Asn Ala Gly
180 185 190

Arg Phe Asp Asp Glu Ile Val Ala Tyr Thr Val Lys Gly Arg Lys Gly
195 200 205

Asp Thr Val Val Asp Lys Asp Glu Tyr Ile Arg His Gly Ala Thr Ile
210 215 220

Glu Gly Met Gln Lys Leu Arg Pro Ala Phe Thr Lys Glu Gly Ser Val
225 230 235 240

Thr Ala Gly Asn Ala Ser Gly Leu Asn Asp Gly Ala Ala Ala Val Met
245 250 255

Val Met Ser Glu Asp Glu Ala Ala Arg Arg Gly Leu Thr Pro Leu Ala
260 265 270

Arg Ile Ala Ser Tyr Ala Thr Ala Gly Leu Asp Pro Ala Ile Met Gly
275 280 285

Thr Gly Pro Ile Pro Ser Ser Arg Lys Ala Leu Glu Lys Ala Gly Trp
290 295 300

Ser Val Gly Asp Leu Asp Leu Val Glu Ala Asn Glu Ala Phe Ala Ala
305 310 315 320

Gln Ala Cys Ala Val Asn Lys Asp Met Gly Trp Asp Pro Ser Ile Val
325 330 335

Asn Val Asn Gly Gly Ala Ile Ala Ile Gly His Pro Ile Gly Ala Ser
340 345 350

Gly Ala Arg Ile Leu Asn Thr Leu Leu Phe Glu Met Gln Arg Arg Asp
355 360 365

Ala Lys Lys Gly Leu Ala Thr Leu Cys Ile Gly Gly Gly Met Gly Val
370 375 380

Ala Met Cys Leu Glu Arg
385 390

<210> 179

<211> 240

<212> PRT

<213> Paracoccus sp. R114

<220>

<221> misc_feature

<222> (1179)..(1194)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<220>

<221> misc_feature

<222> (1196)..(1210)

<223> inverted repeat between genes constituting a putative transcripti
onal sto

<400> 179

```
Met Ser Lys Val Ala Leu Val Thr Gly Gly Ser Arg Gly Ile Gly Ala
1               5                   10                  15

Glu Ile Cys Lys Ala Leu Gln Ala Ala Gly Tyr Thr Val Ala Ala Asn
            20                  25                  30

Tyr Ala Gly Asn Asp Asp Ala Ala Lys Ala Phe Thr Glu Glu Thr Gly
        35                  40                  45

Ile Lys Thr Tyr Lys Trp Ser Val Ala Asp Tyr Asp Ala Cys Lys Ala
    50                  55                  60

Gly Ile Ala Gln Val Glu Glu Asp Leu Gly Pro Ile Ala Val Leu Ile
65                  70                  75                  80

Asn Asn Ala Gly Ile Thr Arg Asp Ala Pro Phe His Lys Met Thr Pro
                85                  90                  95

Glu Lys Trp Lys Glu Val Ile Asp Thr Asn Leu Thr Gly Thr Phe Asn
            100                 105                 110

Met Thr His Pro Val Trp Pro Gly Met Arg Glu Arg Lys Phe Gly Arg
        115                 120                 125

Val Ile Asn Ile Ser Ser Ile Asn Gly Gln Lys Gly Gln Phe Gly Gln
    130                 135                 140

Ala Asn Tyr Ala Ala Ala Lys Ala Gly Asp Leu Gly Phe Thr Lys Ser
145                 150                 155                 160

Leu Ala Gln Glu Gly Ala Arg Asn Asn Ile Thr Val Asn Ala Ile Cys
                165                 170                 175

Pro Gly Tyr Ile Ala Thr Asp Met Val Met Ala Val Pro Glu Gln Val
            180                 185                 190
```

Arg Glu Gly Ile Ile Ala Gln Ile Pro Val Gly Arg Leu Gly Glu Pro
195 200 205

Ser Glu Ile Ala Arg Cys Val Val Phe Leu Ala Ser Asp Asp Ala Gly
210 215 220

Phe Val Thr Gly Ser Thr Ile Thr Ala Asn Gly Gly Gln Tyr Tyr Ile
225 230 235 240

<210> 180

<211> 729

<212> DNA

<213> Paracoccus carotinifaciens E-396

<220>

<221> CDS

<222> (1)..(726)

<223> Beta-carotene Beta-4 oxygenase

<400> 180
atg agc gca cat gcc ctg ccc aag gca gat ctg acc gcc acc agt ttg 48
Met Ser Ala His Ala Leu Pro Lys Ala Asp Leu Thr Ala Thr Ser Leu
1 5 10 15

atc gtc tcg ggc ggc atc atc gcc gcg tgg ctg gcc ctg cat gtg cat 96
Ile Val Ser Gly Gly Ile Ile Ala Ala Trp Leu Ala Leu His Val His
20 25 30

gcg ctg tgg ttt ctg gac gcg gcg gcg cat ccc atc ctg gcg gtc gcg 144
Ala Leu Trp Phe Leu Asp Ala Ala Ala His Pro Ile Leu Ala Val Ala
35 40 45

aat ttc ctg ggg ctg acc tgg ctg tcg gtc ggt ctg ttc atc atc gcg 192
Asn Phe Leu Gly Leu Thr Trp Leu Ser Val Gly Leu Phe Ile Ile Ala
50 55 60

cat gac gcg atg cat ggg tcg gtc gtg ccg ggg cgc ccg cgc gcc aat 240
His Asp Ala Met His Gly Ser Val Val Pro Gly Arg Pro Arg Ala Asn
65 70 75 80

gcg gcg atg ggc cag ctt gtc ctg tgg ctg tat gcc gga ttt tcc tgg 288
Ala Ala Met Gly Gln Leu Val Leu Trp Leu Tyr Ala Gly Phe Ser Trp
85 90 95

```
cgc aag atg atc gtc aag cac atg gcc cat cat cgc cat gcc gga acc      336
Arg Lys Met Ile Val Lys His Met Ala His His Arg His Ala Gly Thr
            100                 105                 110

gac gac gac cca gat ttc gac cat ggc ggc ccg gtc cgc tgg tac gcc      384
Asp Asp Asp Pro Asp Phe Asp His Gly Gly Pro Val Arg Trp Tyr Ala
        115                 120                 125

cgc ttc atc ggc acc tat ttc ggc tgg cgc gag ggg ctg ctg ctg ccc      432
Arg Phe Ile Gly Thr Tyr Phe Gly Trp Arg Glu Gly Leu Leu Leu Pro
    130                 135                 140

gtc atc gtg acg gtc tat gcg ctg atg ttg ggg gat cgc tgg atg tac      480
Val Ile Val Thr Val Tyr Ala Leu Met Leu Gly Asp Arg Trp Met Tyr
145                 150                 155                 160

gtg gtc ttc tgg ccg ttg ccg tcg atc ctg gcg tcg atc cag ctg ttc      528
Val Val Phe Trp Pro Leu Pro Ser Ile Leu Ala Ser Ile Gln Leu Phe
                165                 170                 175

gtg ttc ggc atc tgg ctg ccg cac cgc ccc ggc cac gac gcg ttc ccg      576
Val Phe Gly Ile Trp Leu Pro His Arg Pro Gly His Asp Ala Phe Pro
            180                 185                 190

gac cgc cac aat gcg cgg tcg tcg cgg atc agc gac ccc gtg tcg ctg      624
Asp Arg His Asn Ala Arg Ser Ser Arg Ile Ser Asp Pro Val Ser Leu
        195                 200                 205

ctg acc tgc ttt cac ttt ggc ggt tat cat cac gaa cac cac ctg cac      672
Leu Thr Cys Phe His Phe Gly Gly Tyr His His Glu His His Leu His
    210                 215                 220

ccg acg gtg cct tgg tgg cgc ctg ccc agc acc cgc acc aag ggg gac      720
Pro Thr Val Pro Trp Trp Arg Leu Pro Ser Thr Arg Thr Lys Gly Asp
225                 230                 235                 240

acc gca tga                                                          729
Thr Ala
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<210> 181

<211> 242

<212> PRT

<213> Paracoccus carotinifaciens E-396

<400> 181

```
Met Ser Ala His Ala Leu Pro Lys Ala Asp Leu Thr Ala Thr Ser Leu
1               5                   10                  15
```

Ile Val Ser Gly Gly Ile Ile Ala Ala Trp Leu Ala Leu His Val His
20 25 30

Ala Leu Trp Phe Leu Asp Ala Ala Ala His Pro Ile Leu Ala Val Ala
35 40 45

Asn Phe Leu Gly Leu Thr Trp Leu Ser Val Gly Leu Phe Ile Ile Ala
50 55 60

His Asp Ala Met His Gly Ser Val Val Pro Gly Arg Pro Arg Ala Asn
65 70 75 80

Ala Ala Met Gly Gln Leu Val Leu Trp Leu Tyr Ala Gly Phe Ser Trp
85 90 95

Arg Lys Met Ile Val Lys His Met Ala His His Arg His Ala Gly Thr
100 105 110

Asp Asp Asp Pro Asp Phe Asp His Gly Gly Pro Val Arg Trp Tyr Ala
115 120 125

Arg Phe Ile Gly Thr Tyr Phe Gly Trp Arg Glu Gly Leu Leu Leu Pro
130 135 140

Val Ile Val Thr Val Tyr Ala Leu Met Leu Gly Asp Arg Trp Met Tyr
145 150 155 160

Val Val Phe Trp Pro Leu Pro Ser Ile Leu Ala Ser Ile Gln Leu Phe
165 170 175

Val Phe Gly Ile Trp Leu Pro His Arg Pro Gly His Asp Ala Phe Pro
180 185 190

Asp Arg His Asn Ala Arg Ser Ser Arg Ile Ser Asp Pro Val Ser Leu
195 200 205

Leu Thr Cys Phe His Phe Gly Gly Tyr His His Glu His His Leu His
210 215 220

Pro Thr Val Pro Trp Trp Arg Leu Pro Ser Thr Arg Thr Lys Gly Asp
225 230 235 240

Thr Ala

<210> 182

<211> 510

<212> DNA

<213> Paracoccus sp. R1534

<220>

<221> CDS

<222> (1)..(507)

<223> Beta-Carotene hydroxylase

<400> 182
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atg agc act tgg gcc gca atc ctg acc gtc atc ctg acc gtc gcc gcg      48
Met Ser Thr Trp Ala Ala Ile Leu Thr Val Ile Leu Thr Val Ala Ala
1               5                   10                  15

atg gag ctg acg gcc tac tcc gtc cat cgg tgg atc atg cat ggc ccc      96
Met Glu Leu Thr Ala Tyr Ser Val His Arg Trp Ile Met His Gly Pro
            20                  25                  30

ctg ggc tgg ggc tgg cat aaa tcg cac cac gac gag gat cac gac cac     144
Leu Gly Trp Gly Trp His Lys Ser His His Asp Glu Asp His Asp His
        35                  40                  45

gcg ctc gag aag aac gac ctc tat ggc gtc atc ttc gcg gta atc tcg     192
Ala Leu Glu Lys Asn Asp Leu Tyr Gly Val Ile Phe Ala Val Ile Ser
    50                  55                  60

atc gtg ctg ttc gcg atc ggc gcg atg ggg tcg gat ctg gcc tgg tgg     240
Ile Val Leu Phe Ala Ile Gly Ala Met Gly Ser Asp Leu Ala Trp Trp
65                  70                  75                  80

ctg gcg gtg ggg gtc acc tgc tac ggg ctg atc tac tat ttc ctg cat     288
Leu Ala Val Gly Val Thr Cys Tyr Gly Leu Ile Tyr Tyr Phe Leu His
                85                  90                  95

gac ggc ttg gtg cat ggg cgc tgg ccg ttc cgc tat gtc ccc aag cgc     336
Asp Gly Leu Val His Gly Arg Trp Pro Phe Arg Tyr Val Pro Lys Arg
            100                 105                 110

ggc tat ctt cgt cgc gtc tac cag gca cac agg atg cat cac gcg gtc     384
Gly Tyr Leu Arg Arg Val Tyr Gln Ala His Arg Met His His Ala Val
        115                 120                 125
```

cat ggc cgc gag aac tgc gtc agc ttc ggt ttc atc tgg gcg ccc tcg 432
His Gly Arg Glu Asn Cys Val Ser Phe Gly Phe Ile Trp Ala Pro Ser
130 135 140

gtc gac agc ctc aag gca gag ctg aaa cgc tcg ggc gcg ctg ctg aag 480
Val Asp Ser Leu Lys Ala Glu Leu Lys Arg Ser Gly Ala Leu Leu Lys
145 150 155 160

gac cgc gaa ggg gcg gat cgc aat aca tga 510
Asp Arg Glu Gly Ala Asp Arg Asn Thr
165

<210> 183

<211> 169

<212> PRT

<213> Paracoccus sp. R1534

<400> 183

Met Ser Thr Trp Ala Ala Ile Leu Thr Val Ile Leu Thr Val Ala Ala
1 5 10 15

Met Glu Leu Thr Ala Tyr Ser Val His Arg Trp Ile Met His Gly Pro
20 25 30

Leu Gly Trp Gly Trp His Lys Ser His His Asp Glu Asp His Asp His
35 40 45

Ala Leu Glu Lys Asn Asp Leu Tyr Gly Val Ile Phe Ala Val Ile Ser
50 55 60

Ile Val Leu Phe Ala Ile Gly Ala Met Gly Ser Asp Leu Ala Trp Trp
65 70 75 80

Leu Ala Val Gly Val Thr Cys Tyr Gly Leu Ile Tyr Tyr Phe Leu His
85 90 95

Asp Gly Leu Val His Gly Arg Trp Pro Phe Arg Tyr Val Pro Lys Arg
100 105 110

Gly Tyr Leu Arg Arg Val Tyr Gln Ala His Arg Met His His Ala Val
115 120 125

His Gly Arg Glu Asn Cys Val Ser Phe Gly Phe Ile Trp Ala Pro Ser
130 135 140

Val Asp Ser Leu Lys Ala Glu Leu Lys Arg Ser Gly Ala Leu Leu Lys
145 150 155 160

Asp Arg Glu Gly Ala Asp Arg Asn Thr
165

<210> 184

<211> 888

<212> DNA

<213> Paracoccus sp. R1534

<220>

<221> CDS

<222> (1)..(885)

<223> farnesyltransferase or geranylgeranyl diphosphate synthase

<400> 184
atg acg ccc aag cag caa ttc ccc cta cgc gat ctg gtc gag atc agg 48
Met Thr Pro Lys Gln Gln Phe Pro Leu Arg Asp Leu Val Glu Ile Arg
1 5 10 15

ctg gcg cag atc tcg ggc cag ttc ggc gtg gtc tcg gcc ccg ctc ggc 96
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ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Roche Vitamins Inc.
Attn: Markus Huembelin
340 Kingsland Street
Nutley, NJ 007110-1199

Deposited on Behalf of: Roche Vitamins Inc.

Identification Reference by Depositor: Paracoccus sp.: R-1506

Patent Deposit Designation PTA-3431

The deposit was accompanied by: __ a scientific description _ a proposed taxonomic description indicated above.

The deposit was received June 5, 2001 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 27, 2001. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Tanya Nunnally, Patent Specialist, Patent Depository

Date: June 28, 2001

cc: Kevin C. Hooper
(Ref: Docket or Case No.: C38435/121966)

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Roche Vitamins Inc.
Attn: Markus Huembelin
340 Kingsland Street
Nutley, NJ 07110-1199

Deposited on Behalf of: Roche Vitamins Inc.

Identification Reference by Depositor:	**Patent Deposit Designation**
Paracoccus sp.: R114	PTA-3335
Paracoccus sp.: R1534	PTA-3336

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above. The deposits were received April 24, 2001 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested May 7, 2001. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Tanya Nunnally, Patent Specialist, Patent Depository

Date: May 23, 2001

cc: Kevin C. Hooper
(Ref: Docket or Case No.: C38435/121966)

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International Bureau

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PCT

(10) International Publication Number
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(51) International Patent Classification[7]: C12N 9/02, 9/12, 9/88, 9/90, 15/53, 15/54, 15/60, 15/61, 1/20, 1/21, C12P 7/02, 17/06, C12N 9/10, C12P 23/00

(21) International Application Number: PCT/EP02/06171

(22) International Filing Date: 5 June 2002 (05.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/296,299 6 June 2001 (06.06.2001) US

(71) Applicant (*for all designated States except US*): **ROCHE VITAMINS AG** [CH/CH]; 124 Grenzacherstrasse, CH-4070 Basle (CH).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **BERRY, Alan** [US/CH]; 99 Mattweg, CH-4144 Arlesheim (CH). **BRETZEL, Werner** [DE/DE]; 93B Tuellinger Strasse, 79539 Loerrach (DE). **HUEMBELIN, Markus** [CH/CH]; 2 Wollbacherstrasse, CH-4058 Basle (CH). **LOPEZ-ULIBARRI, Rual** [MX/CH]; 3 Wasserweg, CH-4334 Sisseln (CH). **MAYER, Anne Francoise** [LU/CH]; 100 Rennweg, CH-4052 Basle (CH). **YELISEEV, Alexei** [RU/US]; 1076 Carol Lane, # 118, Lafayette, CA 94549 (US).

(74) Agents: **KELLER, Günter** et al.; Lederer & Keller, Prinzregentenstrasse 16, 80538 München (DE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*

(88) Date of publication of the international search report: 18 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/099095 A3

(54) Title: IMPROVED ISOPRENOID PRODUCTION

(57) Abstract: Isolated polynucleotides encoding polypeptides having the activity of enzymes in the mevalonate pathway, e.g. hydroxymethylglutaryl-CoA reductase, isopetenyl diphosphate isomerase, hydroxymethylglutaryl-CoA synthase, mevolante kinase, phosphomevalonate kinase, or diphosphomevalonate decarboxylase; are provided, useful for recombinantly producing isoprenoid compounds such as carotenoids like phytoene, lycopene, β-carotene, zeaxanthin, canthaxanthin, astaxanthin, adonixanthin, cryptoxanthin, echinenone and adonirubin. Expression vectors, cultured cells, and methods of making isoprenoid compounds are also provided.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/06171

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/02 C12N9/12 C12N9/88 C12N9/90 C12N15/53
C12N15/54 C12N15/60 C12N15/61 C12N1/20 C12N1/21
C12P7/02 C12P17/06 C12N9/10 C12P23/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, SEQUENCE SEARCH, BIOSIS, WPI Data, PAJ, FSTA, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EM_PRO 'Online! EMBL; 2 May 2002 (2002-05-02) HUEMBELIN, M. ET AL.: "Paracoccus zeaxanthinifaciens mvaA, idi, hcs, mvk, pmk, and mvd gene." retrieved from EBI, accession no. AJ431696 Database accession no. PZE431696 XP002232853 abstract ---	1,6

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2003	07.07.2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	De Kok, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EM_PRO 'Online! EMBL; 2 May 2002 (2002-05-02) HUEMBELIN, M. ET AL.: "Paracoccus zeaxanthinifaciens xseB and ispA gene" retrieved from EBI, accession no. PZE431697 Database accession no. AJ431697 XP002242421 abstract	2,3,7,9
P,X	DATABASE EM_PRO 'Online! EMBL; 2 May 2002 (2002-05-02) HUEMBELIN, M.: "Paracoccous zeaxanthinifaciens phaA gene, phaB gene, ORF1 and ddsA gene" retrieved from EBI, accession no. PZE431695 Database accession no. AJ431695 XP002242422 abstract	4,5,8,9
P,X	EISENREICH WOLFGANG ET AL: "Biosynthesis of zeaxanthin via mevalonate in Paracoccus species strain PTA-3335. A product-based retrobiosynthetic study." JOURNAL OF ORGANIC CHEMISTRY, vol. 67, no. 3, 8 February 2002 (2002-02-08), pages 871-875, XP002242419 February 8, 2002 ISSN: 0022-3263 the whole document	16
X	US 3 891 504 A (SCHOCHER ARNO JOHANNES ET AL) 24 June 1975 (1975-06-24) cited in the application the whole document	16
X	WO 00 01650 A (DCV INC) 13 January 2000 (2000-01-13) page 8, line 1 - line 24 page 14, line 12 -page 20, line 27	14
A		1,6, 9-13,15
X	EP 0 747 483 A (HOFFMANN LA ROCHE) 11 December 1996 (1996-12-11) the whole document, especially page 11, lines 10-26.	4,8,16
Y	& US 6 087 152 A 11 July 2000 (2000-07-11) cited in the application	1,6, 9-13,15

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	YABUTANI TETSUYA ET AL: "Analysis of beta-ketothiolase and acetoacetyl-CoA reductase genes of a methylotrophic bacterium, Paracoccus denitrificans, and their expression in Escherichia coli." FEMS MICROBIOLOGY LETTERS, vol. 133, no. 1-2, 1995, pages 85-90, XP002242420 ISSN: 0378-1097 the whole document	4,5,8-13
X	-& DATABASE EM_PRO 'Online! EMBL; 20 February 1995 (1995-02-20) YABUTANI, T. ET AL.: "Paracoccus denitrificans genes for beta-ketothiolase and acetoacetyl-CoA reductase" retrieved from EBI, accession no. PDPHAA Database accession no. D49362 XP002242423 abstract	4,5,8,9
X	-& DATABASE SWALL 'Online! 1 October 1996 (1996-10-01) YABUTANI, T. ET AL.: "Acetoacetyl-CoA-thiolase (phaA)" retrieved from EBI, accession no. THIL-PARDE Database accession no. P54810 XP002242424 abstract ———	4,5,8,9
X	DATABASE SWALL 'Online! 1 October 1996 (1996-10-01) YABUTANI, T. ET AL.: "Acetoacetyl-CoA-reductase (PhaB)" retrieved from EBI, accession no. PHAB_PARDE Database accession no. P50204 XP002242425 abstract ———	4,5,8,9
Y	WILDING E IMOGEN ET AL: "Identification, evolution, and essentiality of the mevalonate pathway for isopentenyl diphosphate biosynthesis in gram-positive cocci." JOURNAL OF BACTERIOLOGY, vol. 182, no. 15, August 2000 (2000-08), pages 4319-4327, XP002232852 ISSN: 0021-9193 abstract ———	1,6, 9-13,15
Y	EP 1 072 683 A (KYOWA HAKKO KOGYO KK) 31 January 2001 (2001-01-31)	2,7
A	page 3, paragraph 20 -page 12, paragraph 160 ———	3,9-15

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 06586 A (YISSUM RES DEV CO) 11 February 1999 (1999-02-11)	1,6, 9-13,15, 16
	the whole document	
Y		2,7
T	HUMBELIN M ET AL: "Genetics of isoprenoid biosynthesis in Paracoccus zeaxanthinifaciens" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 297, no. 1-2, 4 September 2002 (2002-09-04), pages 129-139, XP004388023 ISSN: 0378-1119 the whole document	1,6,9-16
T	DATABASE SWALL 'Online! 1 October 2002 (2002-10-01) HUEMBELIN, H.: "Fragment of deoxyxylulose 5-phosphate synthase" retrieved from EBI, accession no. Q8L1H7 Database accession no. Q8L1H7 XP002242426 abstract	3

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 6, 14-16 completely and claims 9-13 partially

   1.1. Claims: 1, 6, 15 completely and 9-13 partially
   An isolated polypeptide selected from a polypeptide comprising the aminoacid sequence shown in SEQ.ID.No. 43, 45, 47, 49, 51 or 53; a polynucleotide encoding said polypeptides comprising SEQ.ID.No. 24 and their use.

   1.2. Claim : 14
   A method of making a carotenoid-producing cell

   1.3. Claim : 16
   A microorganism of the genus Paracoccus

2. Claims: 2 completely and 7, 9-13 partially

   An isolated polypeptide having the aminoacid sequence shown in SEQ.ID.No. 159; a polynucleotide encoding said polypeptide comprising SEQ.ID.No. 157 and their use.

3. Claims: 3 completely and 7, 9-13 partially

   An isolated polypeptide having the aminoacid sequence shown in SEQ.ID.No. 160; a polynucleotide encoding said polypeptide comprising SEQ.ID.No. 157 and their use.

4. Claims: 4, 5, 8 completely and 9-13 partially

   An isolated polypeptide having the aminoacid sequence shown in SEQ.ID.No. 178 resp. 179; a polynucleotide encoding said polypeptides comprising SEQ.ID.No. 177 and their use.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/06171

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3891504 A	24-06-1975	CH 549094 A	15-05-1974
		AT 304414 B	10-01-1973
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		NL 7110401 A	02-02-1972
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		US 6124113 A	26-09-2000
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